

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
2 August 2007 (02.08.2007)

PCT

(10) International Publication Number
WO 2007/086904 A2

(51) International Patent Classification:
C07K 14/43 (2006.01)

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LS, LT, LU, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SI, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(21) International Application Number:
PCT/US2006/014178

(22) International Filing Date: 12 April 2006 (12.04.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/671,003 13 April 2005 (13.04.2005) US

(71) Applicant (for all designated States except US): **ISIS PHARMACEUTICALS, INC. [US/US]**; 1896 Rutherford Road, Carlsbad, CA 92008 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HALL, Thomas, A. [US/US]; 5239 Wohlford St., Oceanside, CA 92056 (US). SAMPATH, Rangarajan [US/US]; 12223 Mannix Road, San Diego, CA 92129 (US). BLYN, Lawrence [US/US]; 27892 Perales, Mission Viejo, CA 92692 (US).**

(74) Agents: **CASIMIR, David, A. et al.; Melden & Carroll, 101 Howard Street, Suite 305, San Francisco, CA 94105 (US).**

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2007/086904 A2

(54) Title: COMPOSITIONS FOR USE IN IDENTIFICATION OF ADENOVIRUSES

(57) Abstract: The present invention provides compositions, kits and methods for rapid identification and quantification of adenoviruses by molecular mass and base composition analysis.

COMPOSITIONS FOR USE IN IDENTIFICATION OF ADENOVIRUSES

STATEMENT OF GOVERNMENT SUPPORT

[01] This invention was made with United States Government support under DARPA/SPO contracts 4400044016 and 4400076514. The United States Government has certain rights in the invention.

FIELD OF THE INVENTION

[02] The present invention provides compositions, kits and methods for rapid identification and quantification of adenoviruses by molecular mass and base composition analysis.

BACKGROUND OF THE INVENTION

[03] First isolated in 1953 by investigators attempting to establish cell-lines from adenoidal tissue of children removed during tonsillectomy and from military recruits with febrile illness, adenoviruses are a frequent cause of acute upper respiratory tract infections. Adenoviruses are widespread in nature, infecting birds, many mammals and man. There are 2 genera, Aviadenovirus (avian) and Mastadenovirus (mammalian). There are several subgroups of mammalian adenoviruses including: Subgroup A (serotypes 12, 18 and 31), Subgroup B (serotypes 3, 7, 11, 14, 21, 34 and 35), Subgroup C (serotypes 1, 2, 5 and 6), Subgroup D (serotypes 8-10, 13, 15, 17, 19, 20, 22-30, 32, 33 and 36-39), Subgroup E (serotype 4), and Subgroups F-G (serotypes 40 and 41).

[04] All Adenovirus particles are similar: non-enveloped, 60-90 nm diameter and have icosahedral symmetry, containing 252 capsomers: 240 "hexons" + 12 "pentons" at the vertices of the icosahedron (2-3-5 symmetry). Individual protomers can be isolated by progressive chemical disruption of purified virus particles. The hexons consist of a trimer of polypeptide II with a central pore; VI, VIII and IX are minor polypeptides also associated with the hexon, thought to be involved in stabilization and/or assembly of the particle. The pentons, which have a toxin-like activity, are more complex; the base consists of a pentamer of peptide III, 5 molecules of IIIa are also associated with the penton base.

[05] The adenoviral genome consists of linear, non-segmented double-stranded DNA, 30-38 kbp (with size varying among subgroups) which has the theoretical capacity to encode 30-40 genes. The

genomic structure (as determined by cross-hybridization and restriction mapping) is used to assign adenoviruses to subgroups.

[06] Certain types of adenovirus are commonly associated with particular clinical syndromes including: Acute Respiratory Illness, Pharyngitis, Gastroenteritis, Conjunctivitis, Pneumonia, Keratoconjunctivitis, Acute Haemorrhagic Cystitis, and Hepatitis. Most Adenovirus infections involve either the respiratory or gastrointestinal tracts or the eye. Adenovirus infections are very common, most are asymptomatic. Virus can be isolated from the majority of tonsils/adenoids surgically removed, indicating latent infections. It is not known how long the virus can persist in the body, or whether it is capable of reactivation after long periods, causing disease. Adenoviruses are difficult to isolate and populations tend to be heterogeneous among the cells of an infected individual. It is known that virus is reactivated during events of immunosuppression.

[07] The present invention provides, *inter alia*, methods of identifying viruses of the Adenoviridae family. Also provided are oligonucleotide primers, compositions and kits containing the oligonucleotide primers, which define viral bioagent identifying amplicons and, upon amplification, produce corresponding amplification products whose molecular masses provide the means to identify viruses of the Adenoviridae family at the sub-species level.

SUMMARY OF THE INVENTION

[08] The present invention provides compositions, kits and methods for rapid identification and quantification of adenoviruses by molecular mass and base composition analysis.

[09] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 26.

[10] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 121.

[11] Another embodiment is a composition of an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 26 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 121.

[12] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 61.

[13] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 122.

[14] Another embodiment is a composition of is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 61 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 122.

[15] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 38.

[16] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 82.

[17] Another embodiment is a composition of is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 38 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 82.

[18] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 63.

[19] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 95.

[20] Another embodiment is a composition of is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 63 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 95.

[21] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 19.

[22] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 93.

[23] Another embodiment is a composition of is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 19 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 93.

[24] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 54.

[25] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 113.

[26] Another embodiment is a composition of is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 54 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 113.

[27] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 36.

[28] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 98.

[29] Another embodiment is a composition of is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 36 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 98.

[30] One embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 16.

[31] Another embodiment is an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 106.

[32] Another embodiment is a composition of is an oligonucleotide primer pair including an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID

NO: 16 and an oligonucleotide primer 14 to 35 nucleobases in length having at least 70% sequence identity with SEQ ID NO: 106.

[33] In some embodiments, either or both of the primers of the primer pair contain at least one modified nucleobase such as 5-propynyluracil or 5-propynylcytosine for example.

[34] In some embodiments, either or both of the primers of the primer pair comprises at least one universal nucleobase such as inosine for example.

[35] In some embodiments, either or both of the primers of the primer pair comprises at least one non-templated T residue on the 5'-end.

[36] In some embodiments, either or both of the primers of the primer pair comprises at least one non-template tag.

[37] In some embodiments, either or both of the primers of the primer pair comprises at least one molecular mass modifying tag.

[38] Some embodiments are kits that contain one or more of the primer pair compositions. In some embodiments, each member of the one or more primer pairs of the kit is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOS: 61:122, 26:121, 38:82, 63:95, 19:93, 54:113, 36:98 and 16:106. Other kit embodiments may contain one or more of any of the primer pairs listed in Table 2.

[39] Some embodiments are kits that contain a set of two general survey adenovirus primer pairs represented by primer pair compositions wherein each member of each pair of primers has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOS: 61:122, 26:121.

[40] Some embodiments of the kits contain at least one calibration polynucleotide for use in quantitation of adenoviruses in a given sample, and also for use as a positive control for amplification.

[41] Some embodiments of the kits contain at least one anion exchange functional group linked to a magnetic bead.

[42] In some embodiments, the present invention provides primers and compositions comprising pairs of primers, and kits containing the same, and methods for use in identification of adenoviruses. The primers are designed to produce amplification products of DNA encoding genes that have conserved and variable regions across different subgroups and serotypes of adenoviruses.

[43] In some embodiments, the present invention also provides methods for identification of adenoviruses. Nucleic acid from the virus is amplified using the primers described above to obtain an amplification product. The molecular mass of the amplification product is measured. Optionally, the base composition of the amplification product is determined from the molecular mass. The molecular mass or base composition is compared with a plurality of molecular masses or base compositions of known analogous adenovirus identifying amplicons, wherein a match between the molecular mass or base composition and a member of the plurality of molecular masses or base compositions identifies the adenovirus. In some embodiments, the molecular mass is measured by mass spectrometry in a modality such as electrospray ionization (ESI) time of flight (TOF) mass spectrometry or ESI Fourier transform ion cyclotron resonance (FTICR) mass spectrometry, for example. Other mass spectrometry techniques can also be used to measure the molecular mass of adenovirus identifying amplicons.

[44] In some embodiments, the present invention is also directed to a method for determining the presence or absence of an adenovirus in a sample. Nucleic acid from the sample is amplified using the composition described above to obtain an amplification product. The molecular mass of the amplification product is determined. Optionally, the base composition of the amplification product is determined from the molecular mass. The molecular mass or base composition of the amplification product is compared with the known molecular masses or base compositions of one or more known analogous adenovirus identifying amplicons, wherein a match between the molecular mass or base composition of the amplification product and the molecular mass or base composition of one or more known adenovirus identifying amplicons indicates the presence of the adenovirus in the sample. In some embodiments, the molecular mass is measured by mass spectrometry.

[45] In some embodiments, the present invention also provides methods for determination of the quantity of an unknown adenovirus in a sample. The sample is contacted with the composition described above and a known quantity of a calibration polynucleotide comprising a calibration sequence. Nucleic acid from the unknown adenovirus in the sample is concurrently amplified with the composition described above and nucleic acid from the calibration polynucleotide in the sample is concurrently amplified with the composition described above to obtain a first amplification product comprising an adenovirus identifying amplicon and a second amplification product comprising a calibration amplicon. The molecular masses and abundances for the adenovirus identifying amplicon and the calibration

amplicon are determined. The adenovirus identifying amplicon is distinguished from the calibration amplicon based on molecular mass and comparison of adenovirus identifying amplicon abundance and calibration amplicon abundance indicates the quantity of adenovirus in the sample. In some embodiments, the base composition of the adenovirus identifying amplicon is determined.

[46] In some embodiments, the present invention provides methods for detecting or quantifying adenoviruses by combining a nucleic acid amplification process with a mass determination process. In some embodiments, such methods identify or otherwise analyze the adenovirus by comparing mass information from an amplification product with a calibration or control product. Such methods can be carried out in a highly multiplexed and/or parallel manner allowing for the analysis of as many as 300 samples per 24 hours on a single mass measurement platform. The accuracy of the mass determination methods in some embodiments of the present invention permits allows for the ability to discriminate between different adenoviruses such as subgroups A, B, C, D, E, and F, as well as serotypes 3, 4, 7 and 21.

BRIEF DESCRIPTION OF THE DRAWINGS

[47] The foregoing summary of the invention, as well as the following detailed description of the invention, is better understood when read in conjunction with the accompanying drawings which are included by way of example and not by way of limitation.

[48] **Figure 1:** process diagram illustrating a representative primer pair selection process.

[49] **Figure 2:** process diagram illustrating an embodiment of the calibration method.

[50] **Figure 3:** a series of mass spectra of bioagent identifying amplicons obtained by amplification of adenovirus serotypes 21, 12, 8, 7 and 4 with primer pair number 739.

[51] **Figure 4:** a series of mass spectra of amplification products corresponding to calibration amplicons and serotype 4 adenoviral bioagent identifying amplicons produced with primer pair number 769 (SEQ ID NOs: 26:121) with different quantities of genome copies per sample.

[52] **Figure 5:** A representative mass spectrum of amplification products corresponding to adenovirus identifying amplicons and calibration amplicons obtained with primer pair number 943 (SEQ ID NOs: 61:122).

DEFINITIONS

[53] As used herein, the term "abundance" refers to an amount. The amount may be described in terms of concentration which are common in molecular biology such as "copy number," "pfu or plate-forming unit" which are well known to those with ordinary skill. Concentration may be relative to a known standard or may be absolute.

[54] As used herein the term "adenovirus" refers to a virus member of the family *Adenoviridae*. Adenoviruses are classified as group I under the Baltimore classification scheme. Adenoviruses are medium-sized (60-90 nm), non-enveloped icosahedral viruses containing double-stranded DNA. There are 51 immunologically distinct types (6 subgenera: A through F) that can cause human infections. Adenoviruses are unusually stable to chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body and water. Adenoviruses are spread via respiratory droplets.

[55] As used herein, the term "amplifiable nucleic acid" is used in reference to nucleic acids that may be amplified by any amplification method. It is contemplated that "amplifiable nucleic acid" also comprises "sample template."

[56] As used herein the term "amplification" refers to a special case of nucleic acid replication involving template specificity. It is to be contrasted with non-specific template replication (i.e., replication that is template-dependent but not dependent on a specific template). Template specificity is here distinguished from fidelity of replication (i.e., synthesis of the proper polynucleotide sequence) and nucleotide (ribo- or deoxyribo-) specificity. Template specificity is frequently described in terms of "target" specificity. Target sequences are "targets" in the sense that they are sought to be sorted out from other nucleic acid. Amplification techniques have been designed primarily for this sorting out. Template specificity is achieved in most amplification techniques by the choice of enzyme. Amplification enzymes are enzymes that, under conditions they are used, will process only specific sequences of nucleic acid in a heterogeneous mixture of nucleic acid. For example, in the case of Q β replicase, MDV-1 RNA is the specific template for the replicase (D.L. Kacian et al., Proc. Natl. Acad. Sci. USA 69:3038 [1972]). Other nucleic acid will not be replicated by this amplification enzyme. Similarly, in the case of T7 RNA polymerase, this amplification enzyme has a stringent specificity for its own promoters (Chamberlin et al., Nature 228:227 [1970]). In the case of T4 DNA ligase, the enzyme will not ligate the two oligonucleotides or polynucleotides, where there is a mismatch between the oligonucleotide or polynucleotide substrate and the template at the ligation junction (D.Y. Wu and R. B. Wallace, Genomics 4:560 [1989]). Finally, Taq and Pfu polymerases, by virtue of their ability to function at high temperature, are found to display high specificity for the sequences bounded and thus defined by the primers; the high temperature results in thermodynamic conditions that favor primer hybridization with

the target sequences and not hybridization with non-target sequences (H.A. Erlich (ed.), PCR Technology, Stockton Press [1989]).

[57] As used herein, the term "amplification reagents" refers to those reagents (deoxyribonucleotide triphosphates, buffer, etc.), needed for amplification, excluding primers, nucleic acid template, and the amplification enzyme. Typically, amplification reagents along with other reaction components are placed and contained in a reaction vessel (test tube, microwell, etc.).

[58] As used herein, the term "analogous" when used in context of comparison of bioagent identifying amplicons indicates that the bioagent identifying amplicons being compared are produced with the same pair of primers. For example, bioagent identifying amplicon "A" and bioagent identifying amplicon "B", produced with the same pair of primers are analogous with respect to each other. Bioagent identifying amplicon "C", produced with a different pair of primers is not analogous to either bioagent identifying amplicon "A" or bioagent identifying amplicon "B".

[59] As used herein, the term "anion exchange functional group" refers to a positively charged functional group capable of binding an anion through an electrostatic interaction. The most well known anion exchange functional groups are the amines, including primary, secondary, tertiary and quaternary amines.

[60] The term "bacteria" or "bacterium" refers to any member of the groups of eubacteria and archaeabacteria.

[61] As used herein, a "base composition" is the exact number of each nucleobase (for example, A, T, C and G) in a segment of nucleic acid. For example, amplification of nucleic acid of Adenovirus Type 21 with primer pair number 739 produces an amplification product 139 nucleobases in length from nucleic acid of the hexon gene that has a base composition of A36 G31 C44 T28 (by convention - with reference to the sense strand of the amplification product). Because the molecular masses of each of the four natural nucleotides and chemical modifications thereof are known, a measured molecular mass can be deconvoluted to a list of possible base compositions. Identification of a base composition of a sense strand which is complementary to the corresponding antisense strand in terms of base composition provides a confirmation of the true base composition of an unknown amplification product. For example, the base composition of the antisense strand of the 139 nucleobase amplification product described above is A28 G44 C31 T36.

[62] As used herein, a "base composition probability cloud" is a representation of the diversity in base composition resulting from a variation in sequence that occurs among different isolates of a given species. The "base composition probability cloud" represents the base composition constraints for each species and is typically visualized using a pseudo four-dimensional plot.

[63] In the context of this invention, a "bioagent" is any organism, cell, or virus, living or dead, or a nucleic acid derived from such an organism, cell or virus. Examples of bioagents include, but are not limited, to cells, (including but not limited to human clinical samples, bacterial cells and other pathogens), viruses, fungi, protists, parasites, and pathogenicity markers (including but not limited to: pathogenicity islands, antibiotic resistance genes, virulence factors, toxin genes and other bioregulating compounds). Samples may be alive or dead or in a vegetative state (for example, vegetative bacteria or spores) and may be encapsulated or bioengineered. In the context of this invention, a "pathogen" is a bioagent which causes a disease or disorder.

[64] As used herein, a "bioagent division" is defined as group of bioagents above the species level and includes but is not limited to, orders, families, classes, clades, genera or other such groupings of bioagents above the species level.

[65] As used herein, the term "bioagent identifying amplicon" refers to a polynucleotide that is amplified from a bioagent in an amplification reaction and which 1) provides sufficient variability to distinguish among bioagents from whose nucleic acid the bioagent identifying amplicon is produced and 2) whose molecular mass is amenable to a rapid and convenient molecular mass determination modality such as mass spectrometry, for example.

[66] As used herein, the term "biological product" refers to any product originating from an organism. Biological products are often products of processes of biotechnology. Examples of biological products include, but are not limited to: cultured cell lines, cellular components, antibodies, proteins and other cell-derived biomolecules, growth media, growth harvest fluids, natural products and bio-pharmaceutical products.

[67] The terms "biowarfare agent" and "bioweapon" are synonymous and refer to a bacterium, virus, fungus or protozoan that could be deployed as a weapon to cause bodily harm to individuals. Military or terrorist groups may be implicated in deployment of biowarfare agents.

[68] In context of this invention, the term "broad range survey primer pair" refers to a primer pair designed to produce bioagent identifying amplicons across different broad groupings of bioagents. For

example, the ribosomal RNA-targeted primer pairs are broad range survey primer pairs which have the capability of producing bacterial bioagent identifying amplicons for essentially all known bacteria. With respect to broad range primer pairs employed for identification of viruses, a broad range survey primer pair for adenoviruses, such as primer pair number 615 (SEQ ID NOS: 45:101) for example, will produce an adenovirus identifying amplicon for essentially all known members of the *Adenoviridae* family.

[69] The term "calibration amplicon" refers to a nucleic acid segment representing an amplification product obtained by amplification of a calibration sequence with a pair of primers designed to produce a bioagent identifying amplicon.

[70] The term "calibration sequence" refers to a polynucleotide sequence to which a given pair of primers hybridizes for the purpose of producing an internal (i.e: included in the reaction) calibration standard amplification product for use in determining the quantity of a bioagent in a sample. The calibration sequence may be expressly added to an amplification reaction, or may already be present in the sample prior to analysis.

[71] The term "clade primer pair" refers to a primer pair designed to produce bioagent identifying amplicons for species belonging to a clade group. A clade primer pair may also be considered as a "speciating" primer pair which is useful for distinguishing among closely related species.

[72] The term "codon" refers to a set of three adjoined nucleotides (triplet) that codes for an amino acid or a termination signal.

[73] In context of this invention, the term "codon base composition analysis," refers to determination of the base composition of an individual codon by obtaining a bioagent identifying amplicon that includes the codon. The bioagent identifying amplicon will at least include regions of the target nucleic acid sequence to which the primers hybridize for generation of the bioagent identifying amplicon as well as the codon being analyzed, located between the two primer hybridization regions.

[74] As used herein, the terms "complementary" or "complementarity" are used in reference to polynucleotides (i.e., a sequence of nucleotides such as an oligonucleotide or a target nucleic acid) related by the base-pairing rules. For example, for the sequence "5'-A-G-T-3'," is complementary to the sequence "3'-T-C-A-5'." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular

importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids. Either term may also be used in reference to individual nucleotides, especially within the context of polynucleotides. For example, a particular nucleotide within an oligonucleotide may be noted for its complementarity, or lack thereof, to a nucleotide within another nucleic acid strand, in contrast or comparison to the complementarity between the rest of the oligonucleotide and the nucleic acid strand.

[75] The term "complement of a nucleic acid sequence" as used herein refers to an oligonucleotide which, when aligned with the nucleic acid sequence such that the 5' end of one sequence is paired with the 3' end of the other, is in "antiparallel association." Certain bases not commonly found in natural nucleic acids may be included in the nucleic acids of the present invention and include, for example, inosine and 7-deazaguanine. Complementarity need not be perfect; stable duplexes may contain mismatched base pairs or unmatched bases. Those skilled in the art of nucleic acid technology can determine duplex stability empirically considering a number of variables including, for example, the length of the oligonucleotide, base composition and sequence of the oligonucleotide, ionic strength and incidence of mismatched base pairs. Where a first oligonucleotide is complementary to a region of a target nucleic acid and a second oligonucleotide has complementary to the same region (or a portion of this region) a "region of overlap" exists along the target nucleic acid. The degree of overlap will vary depending upon the extent of the complementarity

[76] In context of this invention, the term "division-wide primer pair" refers to a primer pair designed to produce bioagent identifying amplicons within sections of a broader spectrum of bioagents. For example, primer pair number 1113 (SEQ ID NOs: 63:95), a division-wide primer pair, is designed to produce adenovirus identifying amplicons for members of adenovirus subgroup A. Other division-wide primer pairs may be used to produce adenovirus identifying amplicons for other members of adenovirus subgroups including subgroups B, C, D, E and F.

[77] As used herein, the term "concurrently amplifying" used with respect to more than one amplification reaction refers to the act of simultaneously amplifying more than one nucleic acid in a single reaction mixture.

[78] As used herein, the term "drill-down primer pair" refers to a primer pair designed to produce bioagent identifying amplicons for identification of sub-species characteristics or confirmation of a species assignment. For example, primer pair number 200 (SEQ ID NOs: 1:64), a drill-down adenovirus primer pair, is designed to produce adenovirus identifying amplicons for adenovirus serotype 4. Other drill-down primer pairs may be used to produce adenovirus identifying amplicons for other adenovirus serotypes such as, for example, serotypes 3, 7, 16 and 21.

[79] The term "duplex" refers to the state of nucleic acids in which the base portions of the nucleotides on one strand are bound through hydrogen bonding to their complementary bases arrayed on a second strand. The condition of being in a duplex form reflects on the state of the bases of a nucleic acid. By virtue of base pairing, the strands of nucleic acid also generally assume the tertiary structure of a double helix, having a major and a minor groove. The assumption of the helical form is implicit in the act of becoming duplexed.

[80] As used herein, the term "etiology" refers to the causes or origins, of diseases or abnormal physiological conditions.

[81] The term "gene" refers to a DNA sequence that comprises control and coding sequences necessary for the production of an RNA having a non-coding function (e.g., a ribosomal or transfer RNA), a polypeptide or a precursor. The RNA or polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence so long as the desired activity or function is retained.

[82] The terms "homology," "homologous" and "sequence identity" refer to a degree of identity. There may be partial homology or complete homology. A partially homologous sequence is one that is less than 100% identical to another sequence. Determination of sequence identity is described in the following example: a primer 20 nucleobases in length which is otherwise identical to another 20 nucleobase primer but having two non-identical residues has 18 of 20 identical residues ($18/20 = 0.9$ or 90% sequence identity). In another example, a primer 15 nucleobases in length having all residues identical to a 15 nucleobase segment of a primer 20 nucleobases in length would have $15/20 = 0.75$ or 75% sequence identity with the 20 nucleobase primer. In context of the present invention, sequence identity is meant to be properly determined when the query sequence and the subject sequence are both described and aligned in the 5' to 3' direction. Sequence alignment algorithms such as BLAST, will return results in two different alignment orientations. In the Plus/Plus orientation, both the query sequence and the subject sequence are aligned in the 5' to 3' direction. On the other hand, in the Plus/Minus orientation, the query sequence is in the 5' to 3' direction while the subject sequence is in the 3' to 5' direction. It should be understood that with respect to the primers of the present invention, sequence identity is properly determined when the alignment is designated as Plus/Plus. Sequence identity may also encompass alternate or modified nucleobases that perform in a functionally similar manner to the regular nucleobases adenine, thymine, guanine and cytosine with respect to hybridization and primer extension in amplification reactions. In a non-limiting example, if the 5-propynyl pyrimidines propyne C and/or propyne T replace one or more C or T residues in one primer which is otherwise identical to another primer in sequence and length, the two primers will have 100% sequence identity with each other. In

another non-limiting example, Inosine (I) may be used as a replacement for G or T and effectively hybridize to C, A or U (uracil). Thus, if inosine replaces one or more C, A or U residues in one primer which is otherwise identical to another primer in sequence and length, the two primers will have 100% sequence identity with each other. Other such modified or universal bases may exist which would perform in a functionally similar manner for hybridization and amplification reactions and will be understood to fall within this definition of sequence identity.

[83] As used herein, "housekeeping gene" refers to a gene encoding a protein or RNA involved in basic functions required for survival and reproduction of a bioagent. Housekeeping genes include, but are not limited to genes encoding RNA or proteins involved in translation, replication, recombination and repair, transcription, nucleotide metabolism, amino acid metabolism, lipid metabolism, energy generation, uptake, secretion and the like.

[84] As used herein, the term "hybridization" is used in reference to the pairing of complementary nucleic acids. Hybridization and the strength of hybridization (i.e., the strength of the association between the nucleic acids) is influenced by such factors as the degree of complementarity between the nucleic acids, stringency of the conditions involved, and the T_m of the formed hybrid. "Hybridization" methods involve the annealing of one nucleic acid to another, complementary nucleic acid, i.e., a nucleic acid having a complementary nucleotide sequence. The ability of two polymers of nucleic acid containing complementary sequences to find each other and anneal through base pairing interaction is a well-recognized phenomenon. The initial observations of the "hybridization" process by Marmur and Lane, Proc. Natl. Acad. Sci. USA 46:453 (1960) and Doty et al., Proc. Natl. Acad. Sci. USA 46:461 (1960) have been followed by the refinement of this process into an essential tool of modern biology.

[85] The term "*in silico*" refers to processes taking place via computer calculations. For example, electronic PCR (ePCR) is a process analogous to ordinary PCR except that it is carried out using nucleic acid sequences and primer pair sequences stored on a computer formatted medium.

[86] As used herein, "intelligent primers" are primers that are designed to bind to highly conserved sequence regions of a bioagent identifying amplicon that flank an intervening variable region and, upon amplification, yield amplification products which ideally provide enough variability to distinguish individual bioagents, and which are amenable to molecular mass analysis. By the term "highly conserved," it is meant that the sequence regions exhibit between about 80-100%, or between about 90-100%, or between about 95-100% identity among all, or at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% of species or strains.

[87] The "ligase chain reaction" (LCR; sometimes referred to as "Ligase Amplification Reaction" (LAR) described by Barany, Proc. Natl. Acad. Sci., 88:189 (1991); Barany, PCR Methods and Applic., 1:5 (1991); and Wu and Wallace, Genomics 4:560 (1989) has developed into a well-recognized alternative method for amplifying nucleic acids. In LCR, four oligonucleotides, two adjacent oligonucleotides which uniquely hybridize to one strand of target DNA, and a complementary set of adjacent oligonucleotides, that hybridize to the opposite strand are mixed and DNA ligase is added to the mixture. Provided that there is complete complementarity at the junction, ligase will covalently link each set of hybridized molecules. Importantly, in LCR, two probes are ligated together only when they base-pair with sequences in the target sample, without gaps or mismatches. Repeated cycles of denaturation, hybridization and ligation amplify a short segment of DNA. LCR has also been used in combination with PCR to achieve enhanced detection of single-base changes. However, because the four oligonucleotides used in this assay can pair to form two short ligatable fragments, there is the potential for the generation of target-independent background signal. The use of LCR for mutant screening is limited to the examination of specific nucleic acid positions.

[88] The term "locked nucleic acid" or "LNA" refers to a nucleic acid analogue containing one or more 2'-O, 4'-C-methylene- β -D-ribofuranosyl nucleotide monomers in an RNA mimicking sugar conformation. LNA oligonucleotides display unprecedented hybridization affinity toward complementary single-stranded RNA and complementary single- or double-stranded DNA. LNA oligonucleotides induce A-type (RNA-like) duplex conformations.

[89] As used herein, the term "mass-modifying tag" refers to any modification to a given nucleotide which results in an increase in mass relative to the analogous non-mass modified nucleotide. Mass-modifying tags can include heavy isotopes of one or more elements included in the nucleotide such as carbon-13 for example. Other possible modifications include addition of substituents such as iodine or bromine at the 5 position of the nucleobase for example.

[90] The term "mass spectrometry" refers to measurement of the mass of atoms or molecules. The molecules are first converted to ions, which are separated using electric or magnetic fields according to the ratio of their mass to electric charge. The measured masses are used to identify the molecules.

[91] The term "microorganism" as used herein means an organism too small to be observed with the unaided eye and includes, but is not limited to bacteria, virus, protozoans, fungi; and ciliates.

[92] The term "multi-drug resistant" or multiple-drug resistant" refers to a microorganism which is resistant to more than one of the antibiotics or antimicrobial agents used in the treatment of said microorganism.

[93] The term "multiplex PCR" refers to a PCR reaction where more than one primer set is included in the reaction pool allowing 2 or more different DNA targets to be amplified by PCR in a single reaction tube.

[94] The term "non-template tag" refers to a stretch of at least three guanine or cytosine nucleobases of a primer used to produce a bioagent identifying amplicon which are not complementary to the template. A non-template tag is incorporated into a primer for the purpose of increasing the primer-duplex stability of later cycles of amplification by incorporation of extra G-C pairs which each have one additional hydrogen bond relative to an A-T pair.

[95] The term "nucleic acid sequence" as used herein refers to the linear composition of the nucleic acid residues A, T, C or G or any modifications thereof, within an oligonucleotide, nucleotide or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single or double stranded, and represent the sense or antisense strand

[96] As used herein, the term "nucleobase" is synonymous with other terms in use in the art including "nucleotide," "deoxynucleotide," "nucleotide residue," "deoxynucleotide residue," "nucleotide triphosphate (NTP)," or deoxynucleotide triphosphate (dNTP).

[97] The term "nucleotide analog" as used herein refers to modified or non-naturally occurring nucleotides such as 5-propynyl pyrimidines (i.e., 5-propynyl-dTTP and 5-propynyl-dTCP), 7-deaza purines (i.e., 7-deaza-dATP and 7-deaza-dGTP). Nucleotide analogs include base analogs and comprise modified forms of deoxyribonucleotides as well as ribonucleotides.

[98] The term "oligonucleotide" as used herein is defined as a molecule comprising two or more deoxyribonucleotides or ribonucleotides, preferably at least 5 nucleotides, more preferably at least about 13 to 35 nucleotides. The exact size will depend on many factors, which in turn depend on the ultimate function or use of the oligonucleotide. The oligonucleotide may be generated in any manner, including chemical synthesis, DNA replication, reverse transcription, PCR, or a combination thereof. Because mononucleotides are reacted to make oligonucleotides in a manner such that the 5' phosphate of one mononucleotide pentose ring is attached to the 3' oxygen of its neighbor in one direction via a phosphodiester linkage, an end of an oligonucleotide is referred to as the "5'-end" if its 5' phosphate is not

linked to the 3' oxygen of a mononucleotide pentose ring and as the "3'-end" if its 3' oxygen is not linked to a 5' phosphate of a subsequent mononucleotide pentose ring. As used herein, a nucleic acid sequence, even if internal to a larger oligonucleotide, also may be said to have 5' and 3' ends. A first region along a nucleic acid strand is said to be upstream of another region if the 3' end of the first region is before the 5' end of the second region when moving along a strand of nucleic acid in a 5' to 3' direction. All oligonucleotide primers disclosed herein are understood to be presented in the 5' to 3' direction when reading left to right. When two different, non-overlapping oligonucleotides anneal to different regions of the same linear complementary nucleic acid sequence, and the 3' end of one oligonucleotide points towards the 5' end of the other, the former may be called the "upstream" oligonucleotide and the latter the "downstream" oligonucleotide. Similarly, when two overlapping oligonucleotides are hybridized to the same linear complementary nucleic acid sequence, with the first oligonucleotide positioned such that its 5' end is upstream of the 5' end of the second oligonucleotide, and the 3' end of the first oligonucleotide is upstream of the 3' end of the second oligonucleotide, the first oligonucleotide may be called the "upstream" oligonucleotide and the second oligonucleotide may be called the "downstream" oligonucleotide.

[99] In the context of this invention, a "pathogen" is a bioagent which causes a disease or disorder.

[100] As used herein, the terms "PCR product," "PCR fragment," and "amplification product" refer to the resultant mixture of compounds after two or more cycles of the PCR steps of denaturation, annealing and extension are complete. These terms encompass the case where there has been amplification of one or more segments of one or more target sequences.

[101] The term "peptide nucleic acid" ("PNA") as used herein refers to a molecule comprising bases or base analogs such as would be found in natural nucleic acid, but attached to a peptide backbone rather than the sugar-phosphate backbone typical of nucleic acids. The attachment of the bases to the peptide is such as to allow the bases to base pair with complementary bases of nucleic acid in a manner similar to that of an oligonucleotide. These small molecules, also designated anti gene agents, stop transcript elongation by binding to their complementary strand of nucleic acid (Nielsen, et al. Anticancer Drug Des. 8:53 63).

[102] The term "polymerase" refers to an enzyme having the ability to synthesize a complementary strand of nucleic acid from a starting template nucleic acid strand and free dNTPs.

[103] As used herein, the term "polymerase chain reaction" ("PCR") refers to the method of K.B. Mullis U.S. Patent Nos. 4,683,195, 4,683,202, and 4,965,188, hereby incorporated by reference, that

describe a method for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. This process for amplifying the target sequence consists of introducing a large excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence, followed by a precise sequence of thermal cycling in the presence of a DNA polymerase. The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, the mixture is denatured and the primers then annealed to their complementary sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing, and polymerase extension can be repeated many times (i.e., denaturation, annealing and extension constitute one "cycle"; there can be numerous "cycles") to obtain a high concentration of an amplified segment of the desired target sequence. The length of the amplified segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the "polymerase chain reaction" (hereinafter "PCR"). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified." With PCR, it is possible to amplify a single copy of a specific target sequence in genomic DNA to a level detectable by several different methodologies (e.g., hybridization with a labeled probe; incorporation of biotinylated primers followed by avidin-enzyme conjugate detection; incorporation of 32P-labeled deoxynucleotide triphosphates, such as dCTP or dATP, into the amplified segment). In addition to genomic DNA, any oligonucleotide or polynucleotide sequence can be amplified with the appropriate set of primer molecules. In particular, the amplified segments created by the PCR process itself are, themselves, efficient templates for subsequent PCR amplifications.

[104] The term "polymerization means" or "polymerization agent" refers to any agent capable of facilitating the addition of nucleoside triphosphates to an oligonucleotide. Preferred polymerization means comprise DNA and RNA polymerases.

[105] As used herein, the terms "pair of primers," or "primer pair" are synonymous. A primer pair is used for amplification of a nucleic acid sequence. A pair of primers comprises a forward primer and a reverse primer. The forward primer hybridizes to a sense strand of a target gene sequence to be amplified and primes synthesis of an antisense strand (complementary to the sense strand) using the target sequence as a template. A reverse primer hybridizes to the antisense strand of a target gene sequence to be amplified and primes synthesis of a sense strand (complementary to the antisense strand) using the target sequence as a template.

[106] The primers are designed to bind to highly conserved sequence regions of a bioagent identifying amplicon that flank an intervening variable region and yield amplification products which ideally provide enough variability to distinguish each individual bioagent, and which are amenable to molecular mass analysis. In some embodiments, the highly conserved sequence regions exhibit between about 80-100%, or between about 90-100%, or between about 95-100% identity, or between about 99-100% identity. The molecular mass of a given amplification product provides a means of identifying the bioagent from which it was obtained, due to the variability of the variable region. Thus design of the primers requires selection of a variable region with appropriate variability to resolve the identity of a given bioagent. Bioagent identifying amplicons are ideally specific to the identity of the bioagent.

[107] Properties of the primers may include any number of properties related to structure including, but not limited to: nucleobase length which may be contiguous (linked together) or non-contiguous (for example, two or more contiguous segments which are joined by a linker or loop moiety), modified or universal nucleobases (used for specific purposes such as for example, increasing hybridization affinity, preventing non-templated adenylation and modifying molecular mass) percent complementarity to a given target sequences.

[108] Properties of the primers also include functional features including, but not limited to, orientation of hybridization (forward or reverse) relative to a nucleic acid template. The coding or sense strand is the strand to which the forward priming primer hybridizes (forward priming orientation) while the reverse priming primer hybridizes to the non-coding or antisense strand (reverse priming orientation). The functional properties of a given primer pair also include the generic template nucleic acid to which the primer pair hybridizes. For example, identification of bioagents can be accomplished at different levels using primers suited to resolution of each individual level of identification. Broad range survey primers are designed with the objective of identifying a bioagent as a member of a particular division (e.g., an order, family, genus or other such grouping of bioagents above the species level of bioagents). In some embodiments, broad range survey intelligent primers are capable of identification of bioagents at the species or sub-species level. Other primers may have the functionality of producing bioagent identifying amplicons for members of a given taxonomic genus, clade, species, sub-species or genotype (including genetic variants which may include presence of virulence genes or antibiotic resistance genes or mutations). Additional functional properties of primer pairs include the functionality of performing amplification either singly (single primer pair per amplification reaction vessel) or in a multiplex fashion (multiple primer pairs and multiple amplification reactions within a single reaction vessel).

[109] As used herein, the terms "purified" or "substantially purified" refer to molecules, either nucleic or amino acid sequences, that are removed from their natural environment, isolated or separated,

and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated. An "isolated polynucleotide" or "isolated oligonucleotide" is therefore a substantially purified polynucleotide.

[110] The term "reverse transcriptase" refers to an enzyme having the ability to transcribe DNA from an RNA template. This enzymatic activity is known as reverse transcriptase activity. Reverse transcriptase activity is desirable in order to obtain DNA from RNA viruses which can then be amplified and analyzed by the methods of the present invention.

[111] The term "ribosomal RNA" or "rRNA" refers to the primary ribonucleic acid constituent of ribosomes. Ribosomes are the protein-manufacturing organelles of cells and exist in the cytoplasm. Ribosomal RNAs are transcribed from the DNA genes encoding them.

[112] The term "sample" in the present specification and claims is used in its broadest sense. On the one hand it is meant to include a specimen or culture (e.g., microbiological cultures). On the other hand, it is meant to include both biological and environmental samples. A sample may include a specimen of synthetic origin. Biological samples may be animal, including human, fluid, solid (e.g., stool) or tissue, as well as liquid and solid food and feed products and ingredients such as dairy items, vegetables, meat and meat by-products, and waste. Biological samples may be obtained from all of the various families of domestic animals, as well as feral or wild animals, including, but not limited to, such animals as ungulates, bear, fish, lagomorphs, rodents, etc. Environmental samples include environmental material such as surface matter, soil, water, air and industrial samples, as well as samples obtained from food and dairy processing instruments, apparatus, equipment, utensils, disposable and non-disposable items. These examples are not to be construed as limiting the sample types applicable to the present invention. The term "source of target nucleic acid" refers to any sample that contains nucleic acids (RNA or DNA). Particularly preferred sources of target nucleic acids are biological samples including, but not limited to blood, saliva, cerebral spinal fluid, pleural fluid, milk, lymph, sputum and semen.

[113] As used herein, the term "sample template" refers to nucleic acid originating from a sample that is analyzed for the presence of "target" (defined below). In contrast, "background template" is used in reference to nucleic acid other than sample template that may or may not be present in a sample. Background template is often a contaminant. It may be the result of carryover, or it may be due to the presence of nucleic acid contaminants sought to be purified away from the sample. For example, nucleic acids from organisms other than those to be detected may be present as background in a test sample.

[114] A "segment" is defined herein as a region of nucleic acid within a target sequence.

[115] The "self-sustained sequence replication reaction" (3SR) (Guatelli et al., Proc. Natl. Acad. Sci., 87:1874-1878 [1990], with an erratum at Proc. Natl. Acad. Sci., 87:7797 [1990]) is a transcription-based *in vitro* amplification system (Kwok et al., Proc. Natl. Acad. Sci., 86:1173-1177 [1989]) that can exponentially amplify RNA sequences at a uniform temperature. The amplified RNA can then be utilized for mutation detection (Fahy et al., PCR Meth. Appl., 1:25-33 [1991]). In this method, an oligonucleotide primer is used to add a phage RNA polymerase promoter to the 5' end of the sequence of interest. In a cocktail of enzymes and substrates that includes a second primer, reverse transcriptase, RNase H, RNA polymerase and ribo- and deoxyribonucleoside triphosphates, the target sequence undergoes repeated rounds of transcription, cDNA synthesis and second-strand synthesis to amplify the area of interest. The use of 3SR to detect mutations is kinetically limited to screening small segments of DNA (e.g., 200-300 base pairs).

[116] As used herein, the term ""sequence alignment"" refers to a listing of multiple DNA or amino acid sequences and aligns them to highlight their similarities. The listings can be made using bioinformatics computer programs.

[117] In context of this invention, the term "speciating primer pair" refers to a primer pair designed to produce a bioagent identifying amplicon with the diagnostic capability of identifying species members of a group of genera or a particular genus of bioagents. Primer pair number 769 (SEQ ID NOs: 26:121), for example, is a speciating primer pair used to identify subgroup and serotype members of the *Adenoviridae* family.

[118] As used herein, a "sub-species characteristic" is a genetic characteristic that provides the means to distinguish two members of the same bioagent species. For example, one viral strain could be distinguished from another viral strain of the same species by possessing a genetic change (e.g., for example, a nucleotide deletion, addition or substitution) in one of the viral genes, such as the RNA-dependent RNA polymerase. Sub-species characteristics are responsible for the phenotypic differences among the different serotypes of adenoviruses.

[119] As used herein, the term "target," refers to a nucleic acid sequence or structure to be detected or characterized. Thus, the "target" is sought to be sorted out from other nucleic acid sequences and contains a sequence that has at least partial complementarity with an oligonucleotide primer. The target nucleic acid may comprise single- or double-stranded DNA or RNA. A "segment" is defined as a region of nucleic acid within the target sequence.

[120] The term "template" refers to a strand of nucleic acid on which a complementary copy is built from nucleoside triphosphates through the activity of a template-dependent nucleic acid polymerase. Within a duplex the template strand is, by convention, depicted and described as the "bottom" strand. Similarly, the non-template strand is often depicted and described as the "top" strand.

[121] As used herein, the term " T_m " is used in reference to the "melting temperature." The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. Several equations for calculating the T_m of nucleic acids are well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: $T_m=81.5+0.41(\% G+C)$, when a nucleic acid is in aqueous solution at 1 M NaCl (see e.g., Anderson and Young, Quantitative Filter Hybridization, in Nucleic Acid Hybridization (1985). Other references (e.g., Allawi, H. T. & SantaLucia, J., Jr. Thermodynamics and NMR of internal G.T mismatches in DNA. Biochemistry 36, 10581-94 (1997) include more sophisticated computations which take structural and environmental, as well as sequence characteristics into account for the calculation of T_m .

[122] The term "triangulation genotyping analysis" refers to a method of genotyping a bioagent by measurement of molecular masses or base compositions of amplification products, corresponding to bioagent identifying amplicons, obtained by amplification of regions of more than one gene. In this sense, the term "triangulation" refers to a method of establishing the accuracy of information by comparing three or more types of independent points of view bearing on the same findings. Triangulation genotyping analysis carried out with a plurality of triangulation genotyping analysis primers yields a plurality of base compositions that then provide a pattern or "barcode" from which a species type can be assigned. The species type may represent a previously known sub-species or strain, or may be a previously unknown strain having a specific and previously unobserved base composition barcode indicating the existence of a previously unknown genotype.

[123] As used herein, the term "triangulation genotyping analysis primer pair" is a primer pair designed to produce bioagent identifying amplicons for determining species types in a triangulation genotyping analysis.

[124] The employment of more than one bioagent identifying amplicon for identification of a bioagent is herein referred to as "triangulation identification." Triangulation identification is pursued by analyzing a plurality of bioagent identifying amplicons produced with different primer pairs. This process is used to reduce false negative and false positive signals, and enable reconstruction of the origin of hybrid or otherwise engineered bioagents. For example, identification of the three part toxin genes typical

of *B. anthracis* (Bowen et al., J. Appl. Microbiol., 1999, 87, 270-278) in the absence of the expected signatures from the *B. anthracis* genome would suggest a genetic engineering event.

[125] In the context of this invention, the term "unknown bioagent" may mean either: (i) a bioagent whose existence is known (such as the well known bacterial species *Staphylococcus aureus* for example) but which is not known to be in a sample to be analyzed, or (ii) a bioagent whose existence is not known (for example, the SARS coronavirus was unknown prior to April 2003). For example, if the method for identification of coronaviruses disclosed in commonly owned U.S. Patent Serial No. 10/829,826 (incorporated herein by reference in its entirety) was to be employed prior to April 2003 to identify the SARS coronavirus in a clinical sample, both meanings of "unknown" bioagent are applicable since the SARS coronavirus was unknown to science prior to April, 2003 and since it was not known what bioagent (in this case a coronavirus) was present in the sample. On the other hand, if the method of U.S. Patent Serial No. 10/829,826 was to be employed subsequent to April 2003 to identify the SARS coronavirus in a clinical sample, only the first meaning (i) of "unknown" bioagent would apply since the SARS coronavirus became known to science subsequent to April 2003 and since it was not known what bioagent was present in the sample.

[126] The term "variable sequence" as used herein refers to differences in nucleic acid sequence between two nucleic acids. For example, the genes of two different bacterial species may vary in sequence by the presence of single base substitutions and/or deletions or insertions of one or more nucleotides. These two forms of the structural gene are said to vary in sequence from one another. In the context of the present invention, "viral nucleic acid" includes, but is not limited to, DNA, RNA, or DNA that has been obtained from viral RNA, such as, for example, by performing a reverse transcription reaction. Viral RNA can either be single-stranded (of positive or negative polarity) or double-stranded.

[127] The term "virus" refers to obligate, ultramicroscopic, parasites that are incapable of autonomous replication (i.e., replication requires the use of the host cell's machinery). Viruses can survive outside of a host cell but cannot replicate.

[128] The term "wild-type" refers to a gene or a gene product that has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designated the "normal" or "wild-type" form of the gene. In contrast, the term "modified", "mutant" or "polymorphic" refers to a gene or gene product that displays modifications in sequence and or functional properties (i.e., altered characteristics) when compared to the wild-type gene or gene product. It is noted that naturally-occurring mutants can be

isolated; these are identified by the fact that they have altered characteristics when compared to the wild-type gene or gene product.

[129] As used herein, a "wobble base" is a variation in a codon found at the third nucleotide position of a DNA triplet. Variations in conserved regions of sequence are often found at the third nucleotide position due to redundancy in the amino acid code.

DETAILED DESCRIPTION OF EMBODIMENTS

A. Bioagent Identifying Amplicons

[130] The present invention provides methods for detection and identification of unknown bioagents using bioagent identifying amplicons. Primers are selected to hybridize to conserved sequence regions of nucleic acids derived from a bioagent, and which bracket variable sequence regions to yield a bioagent identifying amplicon, which can be amplified and which is amenable to molecular mass determination. The molecular mass then provides a means to uniquely identify the bioagent without a requirement for prior knowledge of the possible identity of the bioagent. The molecular mass or corresponding base composition signature of the amplification product is then matched against a database of molecular masses or base composition signatures. A match is obtained when an experimentally-determined molecular mass or base composition of an analyzed amplification product is compared with known molecular masses or base compositions of known bioagent identifying amplicons and the experimentally determined molecular mass or base composition is the same as the molecular mass or base composition of one of the known bioagent identifying amplicons. Alternatively, the experimentally-determined molecular mass or base composition may be within experimental error of the molecular mass or base composition of a known bioagent identifying amplicon and still be classified as a match. In some cases, the match may also be classified using a probability of match model such as the models described in U.S. Serial No. 11/073,362, which is commonly owned and incorporated herein by reference in entirety. Furthermore, the method can be applied to rapid parallel multiplex analyses, the results of which can be employed in a triangulation identification strategy. The present method provides rapid throughput and does not require nucleic acid sequencing of the amplified target sequence for bioagent detection and identification.

[131] Despite enormous biological diversity, all forms of life on earth share sets of essential, common features in their genomes. Since genetic data provide the underlying basis for identification of bioagents by the methods of the present invention, it is necessary to select segments of nucleic acids which ideally provide enough variability to distinguish each individual bioagent and whose molecular mass is amenable to molecular mass determination.

[132] Unlike bacterial genomes, which exhibit conservation of numerous genes (i.e. housekeeping genes) across all organisms, viruses do not share a gene that is essential and conserved among all virus families. Therefore, viral identification is achieved within smaller groups of related viruses, such as members of a particular virus family or genus. For example, RNA-dependent RNA polymerase is present in all single-stranded RNA viruses and can be used for broad priming as well as resolution within the virus family.

[133] In some embodiments of the present invention, at least one viral nucleic acid segment is amplified in the process of identifying the bioagent. Thus, the nucleic acid segments that can be amplified by the primers disclosed herein and that provide enough variability to distinguish each individual bioagent and whose molecular masses are amenable to molecular mass determination are herein described as bioagent identifying amplicons.

[134] In some embodiments of the present invention, bioagent identifying amplicons comprise from about 45 to about 150 nucleobases (i.e. from about 45 to about 200 linked nucleosides), although both longer and short regions may be used. One of ordinary skill in the art will appreciate that the invention embodies compounds of 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150 nucleobases in length, or any range therewithin.

[135] It is the combination of the portions of the bioagent nucleic acid segment to which the primers hybridize (hybridization sites) and the variable region between the primer hybridization sites that comprises the bioagent identifying amplicon.

[136] In some embodiments, bioagent identifying amplicons amenable to molecular mass determination which are produced by the primers described herein are either of a length, size or mass compatible with the particular mode of molecular mass determination or compatible with a means of providing a predictable fragmentation pattern in order to obtain predictable fragments of a length compatible with the particular mode of molecular mass determination. Such means of providing a predictable fragmentation pattern of an amplification product include, but are not limited to, cleavage with chemical reagents, restriction enzymes or cleavage primers, for example. Thus, in some embodiments, bioagent identifying amplicons are larger than 150 nucleobases and are amenable to

molecular mass determination following restriction digestion. Methods of using restriction enzymes and cleavage primers are well known to those with ordinary skill in the art.

[137] In some embodiments, amplification products corresponding to bioagent identifying amplicons are obtained using the polymerase chain reaction (PCR) that is a routine method to those with ordinary skill in the molecular biology arts. Other amplification methods may be used such as ligase chain reaction (LCR), low-stringency single primer PCR, and multiple strand displacement amplification (MDA). These methods are also known to those with ordinary skill.

B. Primers and Primer Pairs

[138] In some embodiments the primers are designed to bind to conserved sequence regions of a bioagent identifying amplicon that flank an intervening variable region and yield amplification products which provide variability sufficient to distinguish each individual bioagent, and which are amenable to molecular mass analysis. In some embodiments, the highly conserved sequence regions exhibit between about 80-100%, or between about 90-100%, or between about 95-100% identity, or between about 99-100% identity. The molecular mass of a given amplification product provides a means of identifying the bioagent from which it was obtained, due to the variability of the variable region. Thus, design of the primers involves selection of a variable region with sufficient variability to resolve the identity of a given bioagent. In some embodiments, bioagent identifying amplicons are specific to the identity of the bioagent.

[139] In some embodiments, identification of bioagents is accomplished at different levels using primers suited to resolution of each individual level of identification. Broad range survey primers are designed with the objective of identifying a bioagent as a member of a particular division (e.g., an order, family, genus or other such grouping of bioagents above the species level of bioagents). In some embodiments, broad range survey intelligent primers are capable of identification of bioagents at the species or sub-species level.

[140] In some embodiments, drill-down primers are designed with the objective of identifying a bioagent at the sub-species level (including strains, subtypes, variants and isolates) based on sub-species characteristics which may, for example, include single nucleotide polymorphisms (SNPs), variable number tandem repeats (VNTRs), deletions, drug resistance mutations or any other modification of a nucleic acid sequence of a bioagent relative to other members of a species having different sub-species characteristics. Drill-down intelligent primers are not always required for identification at the sub-species level because broad range survey intelligent primers may, in some cases provide sufficient identification resolution to accomplishing this identification objective.

[141] A representative process flow diagram used for primer selection and validation process is outlined in Figure 1. For each group of organisms, candidate target sequences are identified (200) from which nucleotide alignments are created (210) and analyzed (220). Primers are then designed by selecting appropriate priming regions (230) to facilitate the selection of candidate primer pairs (240). The primer pairs are then subjected to *in silico* analysis by electronic PCR (ePCR) (300) wherein bioagent identifying amplicons are obtained from sequence databases such as GenBank or other sequence collections (310) and checked for specificity *in silico* (320). Bioagent identifying amplicons obtained from GenBank sequences (310) can also be analyzed by a probability model which predicts the capability of a given amplicon to identify unknown bioagents such that the base compositions of amplicons with favorable probability scores are then stored in a base composition database (325). Alternatively, base compositions of the bioagent identifying amplicons obtained from the primers and GenBank sequences can be directly entered into the base composition database (330). Candidate primer pairs (240) are validated by testing their ability to hybridize to target nucleic acid by an *in vitro* amplification by a method such as PCR analysis (400) of nucleic acid from a collection of organisms (410). Amplification products thus obtained are analyzed by gel electrophoresis or by mass spectrometry to confirm the sensitivity, specificity and reproducibility of the primers used to obtain the amplification products (420).

[142] Many of the important pathogens, including the organisms of greatest concern as biowarfare agents, have been completely sequenced. This effort has greatly facilitated the design of primers for the detection of unknown bioagents. The combination of broad-range priming with division-wide and drill-down priming has been used very successfully in several applications of the technology, including environmental surveillance for biowarfare threat agents and clinical sample analysis for medically important pathogens.

[143] Synthesis of primers is well known and routine in the art. The primers may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed.

[144] In some embodiments primers are employed as compositions for use in methods for identification of viral bioagents as follows: a primer pair composition is contacted with nucleic acid (such as, for example, DNA from a DNA virus, or DNA reverse transcribed from the RNA of an RNA virus) of an unknown viral bioagent. The nucleic acid is then amplified by a nucleic acid amplification technique, such as PCR for example, to obtain an amplification product that represents a bioagent identifying amplicon. The molecular mass of each strand of the double-stranded amplification product is determined

by a molecular mass measurement technique such as mass spectrometry for example, wherein the two strands of the double-stranded amplification product are separated during the ionization process. In some embodiments, the mass spectrometry is electrospray Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) or electrospray time of flight mass spectrometry (ESI-TOF-MS). A list of possible base compositions can be generated for the molecular mass value obtained for each strand and the choice of the correct base composition from the list is facilitated by matching the base composition of one strand with a complementary base composition of the other strand. The molecular mass or base composition thus determined is then compared with a database of molecular masses or base compositions of analogous bioagent identifying amplicons for known viral bioagents. A match between the molecular mass or base composition of the amplification product and the molecular mass or base composition of an analogous bioagent identifying amplicon for a known viral bioagent indicates the identity of the unknown bioagent. In some embodiments, the primer pair used is one of the primer pairs of Table 2. In some embodiments, the method is repeated using one or more different primer pairs to resolve possible ambiguities in the identification process or to improve the confidence level for the identification assignment.

[145] In some embodiments, a bioagent identifying amplicon may be produced using only a single primer (either the forward or reverse primer of any given primer pair), provided an appropriate amplification method is chosen, such as, for example, low stringency single primer PCR (LSSP-PCR). Adaptation of this amplification method in order to produce bioagent identifying amplicons can be accomplished by one with ordinary skill in the art without undue experimentation.

[146] In some embodiments, the oligonucleotide primers are broad range survey primers which hybridize to conserved regions of nucleic acid encoding the hexon gene of all (or between 80% and 100%, between 85% and 100%, between 90% and 100% or between 95% and 100%) known adenoviruses and produce adenovirus identifying amplicons.

[147] In some cases, the molecular mass or base composition of a viral bioagent identifying amplicon defined by a broad range survey primer pair does not provide enough resolution to unambiguously identify a viral bioagent at or below the species level. These cases benefit from further analysis of one or more viral bioagent identifying amplicons generated from at least one additional broad range survey primer pair or from at least one additional division-wide primer pair. The employment of more than one bioagent identifying amplicon for identification of a bioagent is herein referred to as triangulation identification.

[148] In other embodiments, the oligonucleotide primers are division-wide primers which hybridize to nucleic acid encoding genes of species within a genus of viruses. In other embodiments, the oligonucleotide primers are drill-down primers which enable the identification of sub-species characteristics. Drill down primers provide the functionality of producing bioagent identifying amplicons for drill-down analyses such as strain typing when contacted with nucleic acid under amplification conditions. Identification of such sub-species characteristics is often critical for determining proper clinical treatment of viral infections. In some embodiments, sub-species characteristics are identified using only broad range survey primers and division-wide and drill-down primers are not used.

[149] In some embodiments, the primers used for amplification hybridize to and amplify genomic DNA, DNA of bacterial plasmids, DNA of DNA viruses or DNA reverse transcribed from RNA of an RNA virus.

[150] In some embodiments, the primers used for amplification hybridize directly to viral RNA and act as reverse transcription primers for obtaining DNA from direct amplification of viral RNA. Methods of amplifying RNA to produce cDNA using reverse transcriptase are well known to those with ordinary skill in the art and can be routinely established without undue experimentation.

[151] In some embodiments, various computer software programs may be used to aid in design of primers for amplification reactions such as *Primer Premier 5* (Premier Biosoft, Palo Alto, CA) or *OLIGO* Primer Analysis Software (Molecular Biology Insights, Cascade, CO). These programs allow the user to input desired hybridization conditions such as melting temperature of a primer-template duplex for example. In some embodiments, an *in silico* PCR search algorithm, such as (ePCR) is used to analyze primer specificity across a plurality of template sequences which can be readily obtained from public sequence databases such as GenBank for example. An existing RNA structure search algorithm (Macke et al., Nucl. Acids Res., 2001, 29, 4724-4735, which is incorporated herein by reference in its entirety) has been modified to include PCR parameters such as hybridization conditions, mismatches, and thermodynamic calculations (SantaLucia, Proc. Natl. Acad. Sci. U.S.A., 1998, 95, 1460-1465, which is incorporated herein by reference in its entirety). This also provides information on primer specificity of the selected primer pairs. In some embodiments, the hybridization conditions applied to the algorithm can limit the results of primer specificity obtained from the algorithm. In some embodiments, the melting temperature threshold for the primer template duplex is specified to be 35 °C or a higher temperature. In some embodiments the number of acceptable mismatches is specified to be seven mismatches or less. In some embodiments, the buffer components and concentrations and primer concentrations may be specified and incorporated into the algorithm, for example, an appropriate primer concentration is about 250 nM and appropriate buffer components are 50 mM sodium or potassium and 1.5 mM Mg²⁺.

[152] One with ordinary skill in the art of design of amplification primers will recognize that a given primer need not hybridize with 100% complementarity in order to effectively prime the synthesis of a complementary nucleic acid strand in an amplification reaction. Moreover, a primer may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event. (e.g., for example, a loop structure or a hairpin structure). The primers of the present invention may comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% sequence identity with any of the primers listed in Table 2. Thus, in some embodiments of the present invention, an extent of variation of 70% to 100%, or any range therewithin, of the sequence identity is possible relative to the specific primer sequences disclosed herein. Determination of sequence identity is described in the following example: a primer 20 nucleobases in length which is identical to another 20 nucleobase primer having two non-identical residues has 18 of 20 identical residues ($18/20 = 0.9$ or 90% sequence identity). In another example, a primer 15 nucleobases in length having all residues identical to a 15 nucleobase segment of primer 20 nucleobases in length would have $15/20 = 0.75$ or 75% sequence identity with the 20 nucleobase primer.

[153] Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for UNIX, Genetics Computer Group, University Research Park, Madison WI), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489). In some embodiments, complementarity of primers with respect to the conserved priming regions of viral nucleic acid is between about 70% and about 75% 80%. In other embodiments, homology, sequence identity or complementarity, is between about 75% and about 80%. In yet other embodiments, homology, sequence identity or complementarity, is at least 85%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or is 100%.

[154] In some embodiments, the primers described herein comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 98%, or at least 99%, or 100% (or any range therewithin) sequence identity with the primer sequences specifically disclosed herein.

[155] One with ordinary skill is able to calculate percent sequence identity or percent sequence homology and able to determine, without undue experimentation, the effects of variation of primer sequence identity on the function of the primer in its role in priming synthesis of a complementary strand of nucleic acid for production of an amplification product of a corresponding bioagent identifying amplicon.

[156] In one embodiment, the primers are at least 13 nucleobases in length. In another embodiment, the primers are less than 36 nucleobases in length.

[157] In some embodiments of the present invention, the oligonucleotide primers are 13 to 35 nucleobases in length (13 to 35 linked nucleotide residues). These embodiments comprise oligonucleotide primers 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35 nucleobases in length, or any range therewithin. The present invention contemplates using both longer and shorter primers. Furthermore, the primers may also be linked to one or more other desired moieties, including, but not limited to, affinity groups, ligands, regions of nucleic acid that are not complementary to the nucleic acid to be amplified, labels, etc. Primers may also form hairpin structures. For example, hairpin primers may be used to amplify short target nucleic acid molecules. The presence of the hairpin may stabilize the amplification complex (see e.g., TAQMAN MicroRNA Assays, Applied Biosystems, Foster City, California).

[158] In some embodiments, any oligonucleotide primer pair may have one or both primers with less than 70% sequence homology with a corresponding member of any of the primer pairs of Table 2 if the primer pair has the capability of producing an amplification product corresponding to a bioagent identifying amplicon. In other embodiments, any oligonucleotide primer pair may have one or both primers with a length greater than 35 nucleobases if the primer pair has the capability of producing an amplification product corresponding to a bioagent identifying amplicon.

[159] In some embodiments, the function of a given primer may be substituted by a combination of two or more primers segments that hybridize adjacent to each other or that are linked by a nucleic acid loop structure or linker which allows a polymerase to extend the two or more primers in an amplification reaction.

[160] In some embodiments, the primer pairs used for obtaining bioagent identifying amplicons are the primer pairs of Table 2. In other embodiments, other combinations of primer pairs are possible by combining certain members of the forward primers with certain members of the reverse primers. An example can be seen in Table 2 for two primer pair combinations of forward primer HEX_HAD_-6_18_F (SEQ ID NO: 47), with the reverse primers HEX_HAD_86_105_2_R (SEQ ID NO: 123), HEX_HAD_61_84_R, or (SEQ ID NO: 81). Arriving at a favorable alternate combination of primers in a primer pair depends upon the properties of the primer pair, most notably the size of the bioagent identifying amplicon that would be produced by the primer pair, which should be between about 45 to about 150 nucleobases in length. Alternatively, a bioagent identifying amplicon longer than 150

nucleobases in length could be cleaved into smaller segments by cleavage reagents such as chemical reagents, or restriction enzymes, for example.

[161] In some embodiments, the primers are configured to amplify nucleic acid of a bioagent to produce amplification products that can be measured by mass spectrometry and from whose molecular masses candidate base compositions can be readily calculated.

[162] In some embodiments, any given primer comprises a modification comprising the addition of a non-templated T residue to the 5' end of the primer (i.e., the added T residue does not necessarily hybridize to the nucleic acid being amplified). The addition of a non-templated T residue has an effect of minimizing the addition of non-templated adenosine residues as a result of the non-specific enzyme activity of *Taq* polymerase (Magnuson et al., *Biotechniques*, 1996, 21, 700-709), an occurrence which may lead to ambiguous results arising from molecular mass analysis.

[163] In some embodiments of the present invention, primers may contain one or more universal bases. Because any variation (due to codon wobble in the 3rd position) in the conserved regions among species is likely to occur in the third position of a DNA (or RNA) triplet, oligonucleotide primers can be designed such that the nucleotide corresponding to this position is a base which can bind to more than one nucleotide, referred to herein as a "universal nucleobase." For example, under this "wobble" pairing, inosine (I) binds to U, C or A; guanine (G) binds to U or C, and uridine (U) binds to U or C. Other examples of universal nucleobases include nitroindoles such as 5-nitroindole or 3-nitropyrrole (Loakes et al., *Nucleosides and Nucleotides*, 1995, 14, 1001-1003), the degenerate nucleotides dP or dK (Hill et al.), an acyclic nucleoside analog containing 5-nitroindazole (Van Aerschot et al., *Nucleosides and Nucleotides*, 1995, 14, 1053-1056) or the purine analog 1-(2-deoxy-β-D-ribofuranosyl)-imidazolo-4-carboxamide (Sala et al., *Nucl. Acids Res.*, 1996, 24, 3302-3306).

[164] In some embodiments, to compensate for the somewhat weaker binding by the wobble base, the oligonucleotide primers are designed such that the first and second positions of each triplet are occupied by nucleotide analogs that bind with greater affinity than the unmodified nucleotide. Examples of these analogs include, but are not limited to, 2,6-diaminopurine which binds to thymine, 5-propynyluracil (also known as propynylated thymine) which binds to adenine and 5-propynylcytosine and phenoxazines, including G-clamp, which binds to G. Propynylated pyrimidines are described in U.S. Patent Nos. 5,645,985, 5,830,653 and 5,484,908, each of which is commonly owned and incorporated herein by reference in its entirety. Propynylated primers are described in U.S Pre-Grant Publication No. 2003-0170682, which is also commonly owned and incorporated herein by reference in its entirety. Phenoxazines are described in U.S. Patent Nos. 5,502,177, 5,763,588, and 6,005,096, each of which is

incorporated herein by reference in its entirety. G-clamps are described in U.S. Patent Nos. 6,007,992 and 6,028,183, each of which is incorporated herein by reference in its entirety.

[165] In some embodiments, for broad priming of rapidly evolving RNA viruses, primer hybridization is enhanced using primers containing 5-propynyl deoxy-cytidine and deoxy-thymidine nucleotides. These modified primers offer increased affinity and base pairing selectivity.

[166] In some embodiments, non-template primer tags are used to increase the melting temperature (T_m) of a primer-template duplex in order to improve amplification efficiency. A non-template tag is at least three consecutive A or T nucleotide residues on a primer which are not complementary to the template. In any given non-template tag, A can be replaced by C or G and T can also be replaced by C or G. Although Watson-Crick hybridization is not expected to occur for a non-template tag relative to the template, the extra hydrogen bond in a G-C pair relative to an A-T pair confers increased stability of the primer-template duplex and improves amplification efficiency for subsequent cycles of amplification when the primers hybridize to strands synthesized in previous cycles.

[167] In other embodiments, propynylated tags may be used in a manner similar to that of the non-template tag, wherein two or more 5-propynylcytidine or 5-propynyluridine residues replace template matching residues on a primer. In other embodiments, a primer contains a modified internucleoside linkage such as a phosphorothioate linkage, for example.

[168] In some embodiments, the primers contain mass-modifying tags. Reducing the total number of possible base compositions of a nucleic acid of specific molecular weight provides a means of avoiding a persistent source of ambiguity in determination of base composition of amplification products. Addition of mass-modifying tags to certain nucleobases of a given primer will result in simplification of *de novo* determination of base composition of a given bioagent identifying amplicon from its molecular mass.

[169] In some embodiments of the present invention, the mass modified nucleobase comprises one or more of the following: for example, 7-deaza-2'-deoxyadenosine-5'-triphosphate, 5-iodo-2'-deoxyuridine-5'-triphosphate, 5-bromo-2'-deoxyuridine-5'-triphosphate, 5-bromo-2'-deoxycytidine-5'-triphosphate, 5-iodo-2'-deoxycytidine-5'-triphosphate, 5-hydroxy-2'-deoxyuridine-5'-triphosphate, 4-thiothymidine-5'-triphosphate, 5-aza-2'-deoxyuridine-5'-triphosphate, 5-fluoro-2'-deoxyuridine-5'-triphosphate, O6-methyl-2'-deoxyguanosine-5'-triphosphate, N2-methyl-2'-deoxyguanosine-5'-triphosphate, 8-oxo-2'-deoxyguanosine-5'-triphosphate or thiothymidine-5'-triphosphate. In some embodiments, the mass-modified nucleobase comprises ^{15}N or ^{13}C or both ^{15}N and ^{13}C .

[170] In some embodiments, multiplex amplification is performed where multiple bioagent identifying amplicons are amplified with a plurality of primer pairs. The advantages of multiplexing are that fewer reaction containers (for example, wells of a 96- or 384-well plate) are needed for each molecular mass measurement, providing time, resource and cost savings because additional bioagent identification data can be obtained within a single analysis. Multiplex amplification methods are well known to those with ordinary skill and can be developed without undue experimentation. However, in some embodiments, one useful and non-obvious step in selecting a plurality candidate bioagent identifying amplicons for multiplex amplification is to ensure that each strand of each amplification product will be sufficiently different in molecular mass that mass spectral signals will not overlap and lead to ambiguous analysis results. In some embodiments, a 10 Da difference in mass of two strands of one or more amplification products is sufficient to avoid overlap of mass spectral peaks.

[171] In some embodiments, as an alternative to multiplex amplification, single amplification reactions can be pooled before analysis by mass spectrometry. In these embodiments, as for multiplex amplification embodiments, it is useful to select a plurality of candidate bioagent identifying amplicons to ensure that each strand of each amplification product will be sufficiently different in molecular mass that mass spectral signals will not overlap and lead to ambiguous analysis results.

C Determination of Molecular Mass of Bioagent Identifying Amplicons

[172] In some embodiments, the molecular mass of a given bioagent identifying amplicon is determined by mass spectrometry. Mass spectrometry has several advantages, not the least of which is high bandwidth characterized by the ability to separate (and isolate) many molecular peaks across a broad range of mass to charge ratio (m/z). Thus mass spectrometry is intrinsically a parallel detection scheme without the need for radioactive or fluorescent labels, since every amplification product is identified by its molecular mass. The current state of the art in mass spectrometry is such that less than femtomole quantities of material can be readily analyzed to afford information about the molecular contents of the sample. An accurate assessment of the molecular mass of the material can be quickly obtained, irrespective of whether the molecular weight of the sample is several hundred, or in excess of one hundred thousand atomic mass units (amu) or Daltons.

[173] In some embodiments, intact molecular ions are generated from amplification products using one of a variety of ionization techniques to convert the sample to gas phase. These ionization methods include, but are not limited to, electrospray ionization (ES), matrix-assisted laser desorption ionization (MALDI) and fast atom bombardment (FAB). Upon ionization, several peaks are observed from one sample due to the formation of ions with different charges. Averaging the multiple readings of molecular mass obtained from a single mass spectrum affords an estimate of molecular mass of the bioagent

identifying amplicon. Electrospray ionization mass spectrometry (ESI-MS) is particularly useful for very high molecular weight polymers such as proteins and nucleic acids having molecular weights greater than 10 kDa, since it yields a distribution of multiply-charged molecules of the sample without causing a significant amount of fragmentation.

[174] The mass detectors used in the methods of the present invention include, but are not limited to, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), time of flight (TOF), ion trap, quadrupole, magnetic sector, Q-TOF, and triple quadrupole.

D. Base Compositions of Bioagent Identifying Amplicons

[175] Although the molecular mass of amplification products obtained using intelligent primers provides a means for identification of bioagents, conversion of molecular mass data to a base composition signature is useful for certain analyses. As used herein, "base composition" is the exact number of each nucleobase (A, T, C and G) determined from the molecular mass of a bioagent identifying amplicon. In some embodiments, a base composition provides an index of a specific organism. Base compositions can be calculated from known sequences of known bioagent identifying amplicons and can be experimentally determined by measuring the molecular mass of a given bioagent identifying amplicon, followed by determination of all possible base compositions which are consistent with the measured molecular mass within acceptable experimental error. The following example illustrates determination of base composition from an experimentally obtained molecular mass of a 46-mer amplification product originating at position 1337 of the 16S rRNA of *Bacillus anthracis*. The forward and reverse strands of the amplification product have measured molecular masses of 14208 and 14079 Da, respectively. The possible base compositions derived from the molecular masses of the forward and reverse strands for the *B. anthracis* products are listed in Table 1.

Table 1
Possible Base Compositions for *B. anthracis* 46mer Amplification Product

Calc. Mass Forward Strand	Mass Error Forward Strand	Base Composition of Forward Strand	Calc. Mass Reverse Strand	Mass Error Reverse Strand	Base Composition of Reverse Strand
14208.2935	0.079520	A1 G17 C10 T18	14079.2624	0.080600	A0 G14 C13 T19
14208.3160	0.056980	A1 G20 C15 T10	14079.2849	0.058060	A0 G17 C18 T11
14208.3386	0.034440	A1 G23 C20 T2	14079.3075	0.035520	A0 G20 C23 T3
14208.3074	0.065560	A6 G11 C3 T26	14079.2538	0.089180	A5 G5 C1 T35
14208.3300	0.043020	A6 G14 C8 T18	14079.2764	0.066640	A5 G8 C6 T27
14208.3525	0.020480	A6 G17 C13 T10	14079.2989	0.044100	A5 G11 C11 T19
14208.3751	0.002060	A6 G20 C18 T2	14079.3214	0.021560	A5 G14 C16 T11
14208.3439	0.029060	A11 G8 C1 T26	14079.3440	0.000980	A5 G17 C21 T3

14208.3665	0.006520	A11 G11 C6 T18	14079.3129	0.030140	A10 G5 C4 T27
14208.3890	0.016020	A11 G14 C11 T10	14079.3354	0.007600	A10 G8 C9 T19
14208.4116	0.038560	A11 G17 C16 T2	14079.3579	0.014940	A10 G11 C14 T11
14208.4030	0.029980	A16 G8 C4 T18	14079.3805	0.037480	A10 G14 C19 T3
14208.4255	0.052520	A16 G11 C9 T10	14079.3494	0.006360	A15 G2 C2 T27
14208.4481	0.075060	A16 G14 C14 T2	14079.3719	0.028900	A15 G5 C7 T19
14208.4395	0.066480	A21 G5 C2 T18	14079.3944	0.051440	A15 G8 C12 T11
14208.4620	0.089020	A21 G8 C7 T10	14079.4170	0.073980	A15 G11 C17 T3
-	-	-	14079.4084	0.065400	A20 G2 C5 T19
-	-	-	14079.4309	0.087940	A20 G5 C10 T13

[176] Among the 16 possible base compositions for the forward strand and the 18 possible base compositions for the reverse strand that were calculated, only one pair (shown in **bold**) are complementary base compositions, which indicates the true base composition of the amplification product. It should be recognized that this logic is applicable for determination of base compositions of any bioagent identifying amplicon, regardless of the class of bioagent from which the corresponding amplification product was obtained.

[177] In some embodiments, assignment of previously unobserved base compositions (also known as “true unknown base compositions”) to a given phylogeny can be accomplished via the use of pattern classifier model algorithms. Base compositions, like sequences, vary slightly from strain to strain within species, for example. In some embodiments, the pattern classifier model is the mutational probability model. On other embodiments, the pattern classifier is the polytope model. The mutational probability model and polytope model are both commonly owned and described in U.S. Patent application Serial No. 11/073,362 which is incorporated herein by reference in entirety.

[178] In one embodiment, it is possible to manage this diversity by building “base composition probability clouds” around the composition constraints for each species. This permits identification of organisms in a fashion similar to sequence analysis. A “pseudo four-dimensional plot” can be used to visualize the concept of base composition probability clouds. Optimal primer design requires optimal choice of bioagent identifying amplicons and maximizes the separation between the base composition signatures of individual bioagents. Areas where clouds overlap indicate regions that may result in a misclassification, a problem which is overcome by a triangulation identification process using bioagent identifying amplicons not affected by overlap of base composition probability clouds.

[179] In some embodiments, base composition probability clouds provide the means for screening potential primer pairs in order to avoid potential misclassifications of base compositions. In other embodiments, base composition probability clouds provide the means for predicting the identity of a

bioagent whose assigned base composition was not previously observed and/or indexed in a bioagent identifying amplicon base composition database due to evolutionary transitions in its nucleic acid sequence. Thus, in contrast to probe-based techniques, mass spectrometry determination of base composition does not require prior knowledge of the composition or sequence in order to make the measurement.

[180] The present invention provides bioagent classifying information similar to DNA sequencing and phylogenetic analysis at a level sufficient to identify a given bioagent. Furthermore, the process of determination of a previously unknown base composition for a given bioagent (for example, in a case where sequence information is unavailable) has downstream utility by providing additional bioagent indexing information with which to populate base composition databases. The process of future bioagent identification is thus greatly improved as more BCS indexes become available in base composition databases.

E. Triangulation Identification

[181] In some cases, a molecular mass of a single bioagent identifying amplicon alone does not provide enough resolution to unambiguously identify a given bioagent. The employment of more than one bioagent identifying amplicon for identification of a bioagent is herein referred to as "triangulation identification." Triangulation identification is pursued by determining the molecular masses of a plurality of bioagent identifying amplicons selected within a plurality of housekeeping genes. This process is used to reduce false negative and false positive signals, and enable reconstruction of the origin of hybrid or otherwise engineered bioagents. For example, identification of the three part toxin genes typical of *B. anthracis* (Bowen et al., J. Appl. Microbiol., 1999, 87, 270-278) in the absence of the expected signatures from the *B. anthracis* genome would suggest a genetic engineering event.

[182] In some embodiments, the triangulation identification process can be pursued by characterization of bioagent identifying amplicons in a massively parallel fashion using the polymerase chain reaction (PCR), such as multiplex PCR where multiple primers are employed in the same amplification reaction mixture, or PCR in multi-well plate format wherein a different and unique pair of primers is used in multiple wells containing otherwise identical reaction mixtures. Such multiplex and multi-well PCR methods are well known to those with ordinary skill in the arts of rapid throughput amplification of nucleic acids. In other related embodiments, one PCR reaction per well or container may be carried out, followed by an amplicon pooling step wherein the amplification products of different wells are combined in a single well or container which is then subjected to molecular mass analysis. The combination of pooled amplicons can be chosen such that the expected ranges of molecular masses of individual amplicons are not overlapping and thus will not complicate identification of signals.

F. Codon Base Composition Analysis

[183] In some embodiments of the present invention, one or more nucleotide substitutions within a codon of a gene of an infectious organism confer drug resistance upon an organism which can be determined by codon base composition analysis. The organism can be a bacterium, virus, fungus or protozoan.

[184] In some embodiments, the amplification product containing the codon being analyzed is of a length of about 35 to about 200 nucleobases. The primers employed in obtaining the amplification product can hybridize to upstream and downstream sequences directly adjacent to the codon, or can hybridize to upstream and downstream sequences one or more sequence positions away from the codon. The primers may have between about 70% to 100% sequence complementarity with the sequence of the gene containing the codon being analyzed.

[185] In some embodiments, the codon base composition analysis is undertaken

[186] In some embodiments, the codon analysis is undertaken for the purpose of investigating genetic disease in an individual. In other embodiments, the codon analysis is undertaken for the purpose of investigating a drug resistance mutation or any other deleterious mutation in an infectious organism such as a bacterium, virus, fungus or protozoan. In some embodiments, the virus is an adenovirus identified in a biological product.

[187] In some embodiments, the molecular mass of an amplification product containing the codon being analyzed is measured by mass spectrometry. The mass spectrometry can be either electrospray (ESI) mass spectrometry or matrix-assisted laser desorption ionization (MALDI) mass spectrometry. Time-of-flight (TOF) is an example of one mode of mass spectrometry compatible with the analyses of the present invention.

[188] The methods of the present invention can also be employed to determine the relative abundance of drug resistant strains of the organism being analyzed. Relative abundances can be calculated from amplitudes of mass spectral signals with relation to internal calibrants. In some embodiments, known quantities of internal amplification calibrants can be included in the amplification reactions and abundances of analyte amplification product estimated in relation to the known quantities of the calibrants.

[189] In some embodiments, upon identification of one or more drug-resistant strains of an infectious organism infecting an individual, one or more alternative treatments can be devised to treat the individual.

G. Determination of the Quantity of a Bioagent

[190] In some embodiments, the identity and quantity of an unknown bioagent can be determined using the process illustrated in Figure 2. Primers (500) and a known quantity of a calibration polynucleotide (505) are added to a sample containing nucleic acid of an unknown bioagent. The total nucleic acid in the sample is then subjected to an amplification reaction (510) to obtain amplification products. The molecular masses of amplification products are determined (515) from which are obtained molecular mass and abundance data. The molecular mass of the bioagent identifying amplicon (520) provides the means for its identification (525) and the molecular mass of the calibration amplicon obtained from the calibration polynucleotide (530) provides the means for its identification (535). The abundance data of the bioagent identifying amplicon is recorded (540) and the abundance data for the calibration data is recorded (545), both of which are used in a calculation (550) which determines the quantity of unknown bioagent in the sample.

[191] A sample comprising an unknown bioagent is contacted with a pair of primers that provide the means for amplification of nucleic acid from the bioagent, and a known quantity of a polynucleotide that comprises a calibration sequence. The nucleic acids of the bioagent and of the calibration sequence are amplified and the rate of amplification is reasonably assumed to be similar for the nucleic acid of the bioagent and of the calibration sequence. The amplification reaction then produces two amplification products: a bioagent identifying amplicon and a calibration amplicon. The bioagent identifying amplicon and the calibration amplicon should be distinguishable by molecular mass while being amplified at essentially the same rate. Effecting differential molecular masses can be accomplished by choosing as a calibration sequence, a representative bioagent identifying amplicon (from a specific species of bioagent) and performing, for example, a 2-8 nucleobase deletion or insertion within the variable region between the two priming sites. The amplified sample containing the bioagent identifying amplicon and the calibration amplicon is then subjected to molecular mass analysis by mass spectrometry, for example. The resulting molecular mass analysis of the nucleic acid of the bioagent and of the calibration sequence provides molecular mass data and abundance data for the nucleic acid of the bioagent and of the calibration sequence. The molecular mass data obtained for the nucleic acid of the bioagent enables identification of the unknown bioagent and the abundance data enables calculation of the quantity of the bioagent, based on the knowledge of the quantity of calibration polynucleotide contacted with the sample.

[192] In some embodiments, construction of a standard curve where the amount of calibration polynucleotide spiked into the sample is varied provides additional resolution and improved confidence

for the determination of the quantity of bioagent in the sample. The use of standard curves for analytical determination of molecular quantities is well known to one with ordinary skill and can be performed without undue experimentation.

[193] In some embodiments, multiplex amplification is performed where multiple bioagent identifying amplicons are amplified with multiple primer pairs which also amplify the corresponding standard calibration sequences. In this or other embodiments, the standard calibration sequences are optionally included within a single vector which functions as the calibration polynucleotide. Multiplex amplification methods are well known to those with ordinary skill and can be performed without undue experimentation.

[194] In some embodiments, the calibrant polynucleotide is used as an internal positive control to confirm that amplification conditions and subsequent analysis steps are successful in producing a measurable amplicon. Even in the absence of copies of the genome of a bioagent, the calibration polynucleotide should give rise to a calibration amplicon. Failure to produce a measurable calibration amplicon indicates a failure of amplification or subsequent analysis step such as amplicon purification or molecular mass determination. Reaching a conclusion that such failures have occurred is in itself, a useful event.

[195] In some embodiments, the calibration sequence is comprised of DNA. In some embodiments, the calibration sequence is comprised of RNA.

[196] In some embodiments, the calibration sequence is inserted into a vector that itself functions as the calibration polynucleotide. In some embodiments, more than one calibration sequence is inserted into the vector that functions as the calibration polynucleotide. Such a calibration polynucleotide is herein termed a "combination calibration polynucleotide." The process of inserting polynucleotides into vectors is routine to those skilled in the art and can be accomplished without undue experimentation. Thus, it should be recognized that the calibration method should not be limited to the embodiments described herein. The calibration method can be applied for determination of the quantity of any bioagent identifying amplicon when an appropriate standard calibrant polynucleotide sequence is designed and used. The process of choosing an appropriate vector for insertion of a calibrant is also a routine operation that can be accomplished by one with ordinary skill without undue experimentation.

H. Identification of Adenoviruses

[197] In other embodiments of the present invention, the primer pairs produce bioagent identifying amplicons within stable and highly conserved regions of adenoviruses. The advantage to characterization

of an amplicon defined by priming regions that fall within a highly conserved region is that there is a low probability that the region will evolve past the point of primer recognition, in which case, the primer hybridization of the amplification step would fail. Such a primer set is thus useful as a broad range survey-type primer. In another embodiment of the present invention, the intelligent primers produce bioagent identifying amplicons in a region which evolves more quickly than the stable region described above. The advantage of characterization bioagent identifying amplicon corresponding to an evolving genomic region is that it is useful for distinguishing emerging strain variants.

[198] The present invention also has significant advantages as a platform for identification of diseases caused by emerging viruses such as, for example, members of the Adenoviridae family. The present invention eliminates the need for prior knowledge of bioagent sequence to generate hybridization probes. Thus, in another embodiment, the present invention provides a means of determining the etiology of a virus infection when the process of identification of viruses is carried out in a clinical setting and, even when the virus is a new species never observed before. This is possible because the methods are not confounded by naturally occurring evolutionary variations (a major concern for characterization of viruses which evolve rapidly) occurring in the sequence acting as the template for production of the bioagent identifying amplicon. Measurement of molecular mass and determination of base composition is accomplished in an unbiased manner without sequence prejudice.

[199] Another embodiment of the present invention also provides a means of tracking the spread of adenovirus when a plurality of samples obtained from different locations are analyzed by the methods described above in an epidemiological setting. In one embodiment, a plurality of samples from a plurality of different locations is analyzed with primer pairs which produce bioagent identifying amplicons, a subset of which contains a specific adenovirus. The corresponding locations of the members of the adenovirus-containing subset indicate the spread of the specific virus to the corresponding locations.

I. Kits

[200] The present invention also provides kits for carrying out the methods described herein. In some embodiments, the kit may comprise a sufficient quantity of one or more primer pairs to perform an amplification reaction on a target polynucleotide from a bioagent to form a bioagent identifying amplicon. In some embodiments, the kit may comprise from one to fifty primer pairs, from one to twenty primer pairs, from one to ten primer pairs, or from two to five primer pairs. In some embodiments, the kit may comprise one or more primer pairs recited in Table 2.

[201] In some embodiments, the kit comprises one or more broad range survey primer(s), division wide primer(s), or drill-down primer(s), or any combination thereof. If a given problem involves

identification of a specific bioagent, the solution to the problem may require the selection of a particular combination of primers to provide the solution to the problem. A kit may be designed so as to comprise particular primer pairs for identification of a particular bioagent. A drill-down kit may be used, for example, to distinguish different sub-species types of adenoviruses or genetically engineered adenoviruses. In some embodiments, the primer pair components of any of these kits may be additionally combined to comprise additional combinations of broad range survey primers and division-wide primers so as to be able to identify the adenovirus.

[202] In some embodiments, the kit contains standardized calibration polynucleotides for use as internal amplification calibrants. Internal calibrants are described in commonly owned U.S. Patent Application Serial No: 60/545,425 which is incorporated herein by reference in its entirety.

[203] In some embodiments, the kit comprises a sufficient quantity of reverse transcriptase (if an RNA virus is to be identified for example), a DNA polymerase, suitable nucleoside triphosphates (including alternative dNTPs such as inosine or modified dNTPs such as the 5-propynyl pyrimidines or any dNTP containing molecular mass-modifying tags such as those described above), a DNA ligase, and/or reaction buffer, or any combination thereof, for the amplification processes described above. A kit may further include instructions pertinent for the particular embodiment of the kit, such instructions describing the primer pairs and amplification conditions for operation of the method. A kit may also comprise amplification reaction containers such as microcentrifuge tubes and the like. A kit may also comprise reagents or other materials for isolating bioagent nucleic acid or bioagent identifying amplicons from amplification, including, for example, detergents, solvents, or ion exchange resins which may be linked to magnetic beads. A kit may also comprise a table of measured or calculated molecular masses and/or base compositions of bioagents using the primer pairs of the kit.

[204] In some embodiments, the kit includes a computer program stored on a computer formatted medium (such as a compact disk or portable USB disk drive, for example) comprising instructions which direct a processor to analyze data obtained from the use of the primer pairs of the present invention. The instructions of the software transform data related to amplification products into a molecular mass or base composition which is a useful concrete and tangible result used in identification and/or classification of bioagents. In some embodiments, the kits of the present invention contain all of the reagents sufficient to carry out one or more of the methods described herein.

[205] While the present invention has been described with specificity in accordance with certain of its embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same. In order that the invention disclosed herein may be more efficiently understood, examples

are provided below. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the invention in any manner.

EXAMPLES

Example 1: Design and Validation of Primers that Define Bioagent Identifying Amplicons for Adenoviruses

A. General Process of Primer Design

[206] For design of primers that define adenovirus identifying amplicons, a series of adenovirus genome segment sequences were obtained, aligned and scanned for regions where pairs of PCR primers would amplify products of about 45 to about 150 nucleotides in length and distinguish subgroups and/or individual serotypes from each other by their molecular masses or base compositions. A typical process shown in Figure 1 is employed for this type of analysis.

[207] A database of expected base compositions for each primer region was generated using an *in silico* PCR search algorithm, such as (ePCR). An existing RNA structure search algorithm (Macke et al., Nucl. Acids Res., 2001, 29, 4724-4735, which is incorporated herein by reference in its entirety) has been modified to include PCR parameters such as hybridization conditions, mismatches, and thermodynamic calculations (SantaLucia, Proc. Natl. Acad. Sci. U.S.A., 1998, 95, 1460-1465, which is incorporated herein by reference in its entirety). This also provides information on primer specificity of the selected primer pairs.

Example 2: Selection of Primers that Define Bioagent Identifying Amplicons for Identification of Adenoviruses

[208] Initial primer design began with the design of primer pairs to produce bioagent identifying amplicons representing segments of the adenoviral hexon gene. These primer pairs were designed to perform a variety of tasks ranging from the general detection of all adenovirus strains to the identification of specific serotypes. Because, in some embodiments, base composition is the final analysis product, one primer pair can be used to identify many serotypes provided that the amplified region has sufficient variation (one base change or more). At the conclusion of the testing phase, a two primer pair test set was selected. These 2 primer pairs (primer pair nos: 943 (SEQ ID NOS: 61:122) and 769 (SEQ ID NOS: 26:121) produce amplicons whose base compositions specifically demonstrate the presence of adenovirus and, in most cases, are simultaneously diagnostic for the serotype of the adenovirus species present. In cases where the two primer pairs cannot specifically identify the serotype of the adenovirus present, other primers can be used to determine the information, such as, for example, any or all of primer pair numbers

1113 (SEQ ID NOs: 38:82), 1117 (SEQ ID NOs: 63:95), 1119 (SEQ ID NOs: 19:93), 1121 (SEQ ID NOs: 54:113), 1124 (SEQ ID NOs: 36:98), and 1126 (SEQ ID NOs: 16:106).

[209] Table 2 represents a collection of primers (sorted by primer pair number) designed to identify adenoviruses using the methods described herein. Tp = 5-propynyluracil; Cp = 5-propynylcytosine. The primer pair number is an in-house database index number. Primer sites were identified on essential adenoviral genes, such as, for example, the hexon gene. The forward or reverse primer name shown in Table 2 indicates the gene region of the viral genome to which the primer hybridizes relative to a reference sequence. In Table 2, for example, the forward primer name HEX_HAD4_1442_1466_F indicates that the forward primer (_F) hybridizes to residues 1442-1466 of an adenovirus reference sequence represented by GenBank Accession No. X84646. GenBank Accession Numbers for reference sequences of the various serotypes of adenoviruses are shown in Table 3 (below) which is sorted according to primer pair number. In some cases, the reference sequences are extractions from adenovirus genomic sequences. One with ordinary skill knows how to obtain individual gene sequences or portions thereof from genomic sequences present in GenBank.

Table 2: Primer Pairs for Identification of Adenoviruses

Primer Pair Number	Forward Primer Name	Forward Sequence	Forward Primer ID No.	Reverse Primer Name	Reverse Sequence	Reverse SEQ ID No.
194	HEX_HAD7+4+21_914_F	AGACCCGAAATTCACTGGTT	2	HEK_HAD7+4+21_976_R	CCAGTCGCTGTGTGATCTAT	68
195	HEX_HAD7+4+21_976_F	ATGTAATCAACGACTGG	5	HEK_HAD7+4+21_1030_R	CAAGTCACACAGGATCA	66
196	HEX_HAD7+4+21_970_F	GCGCTTATGACTACACAG	11	HEK_HAD7+4+21_1039_R	TCTCTCTTGTAGTCGACAC	104
197	HEX_HAD7+3_-771_F	GRAGTTTTTGAGATGAGA	10	HEK_HAD7+3_-809_R	TAATGAGCAATTGAGCGG	76
198	HEX_HAD7+16_-746_-765_F	TGATCTGGCTCTCTTGAC	22	HEX_HAD4+16_828_R	ATAGATGATCTGGCTGC	65
199	HEX_HAD7_505_520_F	GGAGAGCATATCTGACAA	9	HEX_HAD7_559_R	CCACATGAGCTCTGACTG	67
200	HEX_HAD4_-216_-1234_F	ACAGACATTCAAGGGT	1	HEK_HAD4_-1270_R	AATGGATGCTTCATCTGGTC	64
201	HEX_HAD21_515_536_F	TGACTTAAAGAAGAAGGTC	27	HEK_HAD21_547_R	GGCTTGCGCTCTGTTATTC	73
202	HEX_HAD_-1342_-1362_M	CGGATTCGAGTAACTCTGG	7	HEK_HAD_-1446_-1469_ML_R	GGATGATGCTCTAGGGG	75
203	HEX_HAD7+4+21_914_F	AGAAGGCAATTGAAATGGCTT	2	HEK_HAD7+4+21_976_R	CtGCAATGTCGCTGTTGATCAT	70
204	HEX_HAD7+4+21_976_F	ATGGTATCTAACACATGCTGG	5	HEK_HAD7+4+21_1030_R	CAAGTCGCAACAGGATCTGCA	66
205	HEX_HAD7+4+21_970_F	GGGGCTTTTATTTTCTACAGG	11	HEK_HAD7+4+21_1039_R	TCTCTTGTGATGTTGATGACAC	104
206	HEX_HAD7+3_-771_F	GGAAATTGTTTTGATGGTAA	10	HEK_HAD7+3_-809_R	TAATGAGCAATTGTTGAGGG	76
207	HEX_HAD7+16_-746_-765_P	TGATCTGGCTCTCTTGAC	22	HEX_HAD4+16_-828_R	ATATGAGTATCTGTTGATGTTG	65
208	HEX_HAD_-1342_-1362P_F	CGGATTCAGGTTTATCTTGG	7	HEK_HAD_-1446_-1469_NL_R	GATGATGATCTGAGGTTTGTG	75
214	HEX_HAD2_-772_-795_F	CAATTCCGTTCTGGTTCGATAAA	6	HEK_HAD2_-842_R	CTTCGCCGCTCTGAGAGGTTG	72
216	HEX_HAD7+4+21_-73_-90_F	AgtCCGGGCTGGGTCGAG	3	HEK_HAD7+4+21_-163_-179_R	CGGGTCGCGGTCGACATTC	69
217	HEX_HAD7+4+21_1_18_F	ATGGCCACCCATCGATG	4	HEK_HAD7+4+21_-36_-54_R	CTGGTCGCGGCAATGCTG	71
218	HEU_HAD7+4+21_1612_F	GGCTCTTATGTCCTTTCGAGT	12	HEK_HAD7+4+21_-1694_-1718_R	TCTCTTGTGAGTTGCTCTGG	99
613	HEX_HAD_-3_-17_F	GATATGGCCACCCCTGGAT	8	HEK_HAD_-80_R	GGGGGAACTGACGACGAC	74
614	HEX_HAD_-4_-17_F	TGATATGGCCACCCCTGGAT	45	HEK_HAD_82_R	TGGGGCAACTGACGACG	119
615	HEX_HAD_-4_-17P_F	TGATATGGCCACCCCTGGAT	45	HEK_HAD_-82_R	TGGGGCAACTGACGACG	102

DOCKET NO.: DIBS-0073WO (COUNSEL DOCKET NO: 10592)

616	HEX_HAD_-4_-18P_F	TGATATGGCACCCCATGATG	4.6	HEX_HAD_86_101P_R	TGCGCGGGGAGACTGCCA	100
617	HEX_HAD_24_-45P_F	TCTATGGCATTCATGGGACATC	24	HEX_HAD_82_98P_R	TGGCGAACTTCACTGGPAG	118
618	HEX_HAD_1620_1643_F	TGGCTCTTCAATACAGCTGTC	58	HEX_HAD_1702_1727_R	TGTCACATCTTGAGTGTCCA	128
619	HEX_HAD_537_556_F	TGAGCAGACGGCAGATGAG	28	HEX_HAD_635_654_R	TGGCGAGGCGCTGGAC	112
620	HEX_HAD_558_677P_F	TGATCTTCACTGGGTTTGC	29	HEX_HAD_760_786P_R	TGGCGAGGCGCTGGAC	112
621	HEX_HAD_-630_650P_F	TACCTTCACTTACGGCTCA	18	HEX_HAD_724_744P_R	TGATGTTGAACTTCACTGGTAT	96
622	HEX_HAD_-715_713P_F	TCAAGATGGTGTGCTGGC	23	HEX_HAD_745_767P_R	TGACGGTTGCTGGCTTCGAAGTC	105
623	HEX_HAD_-1214_-1212P_F	TGACAGACGCTTACCAAGG	42	HEX_HAD_1318_1339P_R	TGATTTGGCGACGAAAGATT	103
624	HEX_HAD_-799_815P_F	TGACGGCTGGGGTACCA	16	HEX_HAD_895_919P_R	TGATTTGGCGACGAAAGATT	108
625	HEX_HAD_741_765P_E	TGATATGGTGGCTTGTGTTTG	43	HEX_HAD_828_849P_R	TGATTTGGCGACGAAAGATT	106
626	HEX_HAD_-1215_-1214P_F	TGACAGACGCTTACCAAGG	17	HEX_HAD_1277_1290P_R	TGATTTGGCGACGAAAGATT	87
627	HEX_HAD_770_791P_F	TGATATGGTGGCTGGAGAAA	53	HEX_HAD_809_828P_R	TGCTGGTGTCTGGTGTGTC	80
628	HEX_HAD_507_529P_F	TGATGGAGACGATCTACGACACA	62	HEX_HAD_559_578P_R	TGACTTGAGGCTCTGCTG	126
629	HEX_HAD_150_536P_F	TGATATGGTGGCTGGAGGCT	44	HEX_HAD2_547_567P_R	TGCTGGTGTCTGGTGTGTC	114
630	HEX_HAD_730_959P_F	TGACAGACGCTTACGGACGCTT	15	HEX_HAD7_976_997P_R	TGCTGGTGTCTGGTGTGTC	111
631	HEX_HAD_972_996P_F	A	39	HEX_HAD7_1031_1059P_R	TGCGAAGCTGACACAGATCA	125
632	HEX_HAD_966_989P_F	TGCGGGCTGGCTGTTACGACAG	57	HEX_HAD7_1031_1059P_R	TGCTGGTGTCTGGTGTGTC	104
633	HEX_HAD_756_-784P_F	TGATGGAGACGATCTACGACACA	59	HEX_HAD7_917_946P_1_R	TGATGTTGGTGTGTC	77
634	HEX_HAD_730_959P_F	TGATATGGTGGCTGGAGGCT	56	HEX_HAD7_760_787P_1_R	TGATGTTGGTGTGTC	79
635	HEX_HAD7_-1197_-1223P_F	TGCTGGTGTCTGGTGTGTC	33	HEX_HAD7_1301_1330P_1_R	TGATGTTGGTGTGTC	89
640	-1_F	TGG	58	HEX_HAD_1702_1734_R	TGAGCAGCTGGTACATCTGGAG	85
641	HEX_HAD_1620_1643_F	TGTCGCTTCAATACAGCTGCC	45	HEX_HAD_82_9_9_R	TGCGCGGAACTGGACAGCAG	102
707	HEX_HAD_-4_-17_2_F	TGATATGGCACCCATGAT	24	HEX_HAD_82_9_9_R	TGCGCGGAACTGGACAGCAG	118
708	HEX_HAD_24_45_F	TGATGGGGATGAGGAGCATC	24	HEX_HAD_760_786_R	TGCGCGGAACTGGACAGCAG	94
709	HEX_HAD_558_677_F	TGATCTTACGGCTTGGTGC	29	HEX_HAD_724_744_R	TGCGCGGAACTGGACAGCAG	105
710	HEX_HAD_630_650_F	TACCTTCACTGGCTACCA	18	HEX_HAD_745_767_R	TGCGCGGAACTGGACAGCAG	103
711	HEX_HAD_695_713_F	TGACAGCTGGCTGGTC	23			
712	HEX_HAD_-1214_-1212_F	TGACAGACGCTTACCAAGG	42	HEX_HAD_1318_1339_R	TGATTTGGCGACGAAAGATT	109
714	HEX_HAD_930_955_F	TGAGCAGCTGGCTGGTGTGTC	15	HEX_HAD7_976_997_R	TGCGCGGAACTGGACAGCAG	111
715	HEX_HAD_972_996_F	TGCTGGTGTCTGGTGTGTC	39	HEX_HAD7_1031_1059_P_R	TGCGCGGAACTGGACAGCAG	110
716	HEX_HAD7_966_989_F	TGTCGCTTCAATACAGCTACAG	57	HEX_HAD7_1039_1059_R	TGCGCGGAACTGGACAGCAG	104

DOCKET NO.: DIBS-0073WO (COUNSEL DOCKET NO. 10592)

717	HEX_HAD7_756_784_1_F	TGAGATATAGACGCCGGTTCTTG	59	HEK_HAD7_817_846_1_R	T	TAGCTGACATTTCTCTGACATCA	78		
718	HEX_HAD7_650_677_1_F	TGGCTGAAATCCCTTGTGGTCATTC	56	HEK_HAD7_760_787_1_R	TACTNCAGAGAGCAGGCATATTC	79			
719	HEX_HAD7_1197_1223_1_F	TCTTAACTTGTTCCTCGGTG	34	HEK_HAD7_1301_1330_1_R	TGACTCTGGCTCATGGTATTGTTATT	89			
720	HEX_HAD7_971_995_P_F	TCTTGTGGTACTTCACAGGAGTGTG	51	HEK_HAD7_1031_1052_P_R	TGGCTGGTCTCACAGGATTTCA	125			
721	HEX_HAD4_1418_1466_F	TCTTGTGGTACTTCACAGGACTGG	50	HEK_HAD7_1031_1052_R	TGCACTTGACACAGGATCTCA	110			
739	HEX_HAD4_1446_1466_F	TCTAACACACCTCACTGGATGAT	30	HEK_HAD4_1558_1576_R	TGGGGTGGTGGTGTGTTGTT	101			
740	HEX_HAD4_1013_1037_F	TAAACACTACAGCATGAGG	14	HEK_HAD4_1552_1571_R	TGGGGTGGTGGTGTGTTGAGG	127			
741	HEX_HAD4_1013_1041_F	TGATGGTGTGGTGTATCTGAGAC	40	HEK_HAD4_1106_1130_R	TGCTGTTCTACCCCTGATTCACCA	84			
742	HEX_HAD4_1446_1466_F	AAG	41	HEK_HAD4_1106_1130_2_R	TGCTGTTCTACCCCTGATTCACCA	83			
743	HEX_HAD4_1442_1465_F	TGACTACACAGCATGAGTATGG	20	HEK_HAD4_1021_1045_R	TGTTCTGTTCTACCCCTGATTCACCA	124			
768	HEX_HAD4_1442_1466_F	TGACACACACTCCAGTACATGA	25	HEK_HAD4_1538_1562_R	TGTTCTGTTCTACCCCTGATTCACCA	120			
769	HEX_HAD7_1137_1223_P	TGCTTAACTTGTCTTGTCTGAA	26	HEK_HAD4_1537_1562_R	TGTTCTGTTCTACCCCTGATTCACCA	121			
901	HEX_HAD_7_1197_1223_P	TGG	33	HEK_HAD7_1301_1330_P_R	TGACTCTGGTCTCACAGGATTTGG	89			
943	HEX_HAD_7_7_17_F	TGCGAATGATGCCACCCCTGAT	61	HEK_HAD_86_105_R	TGGGGGGGGGGGGGGGGGGGG	122			
944	HEX_HAD_20_45_F	TGCCCGAATGGGATCATGACATC	49	HEK_HAD_86_103_R	TGGGGGGGGGGGGGGGGGGGG	116			
945	HEX_HAD_20_45_F	TGCCCGAATGGGATCATGACATC	49	HEK_HAD_86_105_R	TGGGGGGGGGGGGGGGGGGGG	122			
946	HEX_HAD_-6_18_F	TGCGAATGATGCCACCCCTGATG	47	HEK_HAD_86_105_2_R	TGCGAATGATGCCACCCCTGATG	123			
947	HEX_HAD_-6_18_F	TGCGAATGATGCCACCCCTGATG	47	HEK_HAD_61_84_R	TGACCGGGGAACTGAGTACTCGGA	81			
948	HEX_HAD_22_45_F	TGCCCGAATGGGATCATGACATC	31	HEK_HAD_82_99_R	TGGGGGGGGGGGGGGGGGGGG	102			
1113	HEX_HAD_512_556_F	TGCTTACACACACACACACACAC	39	HEK_HAD_635_655_R	TTGGTCTGAGGACGACGCG	82			
1114	HEX_HAD_1989_2010_F	TGCTTACACACACACACACAC	32	HEK_HAD_2060_2086_R	TGAGTGTATGATGATGATGATGATC	115			
1115	HEX_HAD_2055_2084_F	TGG	13	HEK_HAD_2129_2156_R	TCCCTCATCCTGAGACACCTTATTGCA	97			
1116	HEX_HAD8_656_677_F	TGGCATGTTGGGCGCTTGTG	48	HEK_HADB_760_790_R	TCCACCTCATGAGATTCATGTCGAT	92			
1117	HEX_HADC_1783_1810_F	TGAGACCTGAGGAGGTTAACAT	63	HEK_HADB_1702_1730_R	TCCATGTTCTACCTCTTGTGAGTTCC	95			
1118	HEX_HADC_1537_1562_F	GG	52	HEK_HADC_1800_1949_R	TGGCGGTGTGGGGCGAT	86			
1119	HEX_HADC_1416_1463_F	TGCGACTACAGACAGCGAGGTG	19	HEK_HADC_1642_1662_R	TGAGACATGTTGGGGCGAT	93			
1120	HEX_HADC_1416_1463_F	TCTCTGGGAAATTCCTACTCC	35	HEK_HADC_1552_1570_R	TGGGGGGGGGGGGGGGGGGGG	117			

DOCKET NO.: DIBIS-0073WO (COUNSEL DOCKET NO.: 10592)

	P	AA		
1121	HEX_HA0D_693_713_F	TGGCAAGCTGGTGTGCG	54	TGCTCGATATTCTCTGTCAGTC
1122	HEX_HA0D_621_646_F	TTCATGCCAACAGACCAACTACA	60	TGACCAAGCCAGCACCCATGT
1123	HEX_HA0D_1208_1232_F	TGGATGAGCAGATACTTAACGG	55	TGATTCATAGCAGAAGGATT
1124	HEX_HA0D_665_689_F	TCCOCOMGCCTACACAGACG	36	TCCCTAATTTGCTGTCAGAAGCCA
1125	HEX_HA0D_599_625_F	TATGAGAAATATGAGGAAGCT	21	TGACCTCTTGTTGTTGAGCTTGCG
1126	HEX_HA0D_799_815_F	TAAGCCGCTATACCA	16	TGAGATTTCTAAACCAATGTA
1127	HEX_HA0F_1847_1869_F	TCTACCTCTGGCTGCAAGAAG	37	TGACCCCATTTGGAGGATGAA
				91

Table 3: Reference Sequence Details for Primer Pair Name Coordinates

Primer Pair No.	Reference Sequence Adenovirus Type	Source for Reference Sequence (GenBank Accession No.)	Reference SEQ ID NO:	Specificity (Adenovirus Groups and Types)
194	7	Z48571	129	Types 7, 4 and 21
195	7	Z48571	129	Types 7, 4 and 21
196	7	Z48571	129	Types 7, 4 and 21
197	7	Z48571	129	Types 7 and 3
198	4	X84646	130	Types 4 and 16
199	7	Z48571	129	Type 7
200	4	X84646	130	Type 4
201	21	AY008279	131	Type 21
202	7	Z48571	129	All
203	7	Z48571	129	Types 7, 4 and 21
204	7	Z48571	129	Types 7, 4 and 21
205	7	Z48571	129	Types 7, 4 and 21
206	7	Z48571	129	Types 7 and 3
207	4	X84646	130	Types 4 and 16
208	7	Z48571	129	All
214	2	AJ278924	132	Type 2
216	7	Z48571	129	Types 7, 4 and 21
217	7	Z48571	129	Types 7, 4 and 21
218	7	Z48571	129	Types 7, 4 and 21
613	7	Z48571	129	All
614	7	Z48571	129	All
615	7	Z48571	129	All
616	7	Z48571	129	All
617	7	Z48571	129	All
618	7	Z48571	129	All
619	12	X73487	133	Adenovirus A
620	7	Z48571	129	Adenovirus B
621	1	AF534906	134	Adenovirus C
622	8	AB090341	135	Adenovirus D
623	4	X84646	130	Adenovirus E
624	40	L19443	136	Adenovirus F
625	4	X84646	130	Types 4 and 16
626	4	X84646	130	Type 4

627	7	Z48571	129	Types 3 and 7
628	7	Z48571	129	Type 21
629	21	AY008279	131	Type 21
630	7	Z48571	129	Types 3, 4, 7 and 21
631	7	Z48571	129	Types 3, 4, 7 and 21
632	7	Z48571	129	Types 3, 4, 7 and 21
638	7	Z48571	129	Types 3, 4, 7 and 21
639	7	Z48571	129	Types 3, 4, 7 and 21
640	7	Z48571	129	Types 3, 4, 7 and 21
641	7	Z48571	129	All
707	7	Z48571	129	All
708	7	Z48571	129	All
709	7	Z48571	129	Adenovirus B
710	1	AF534906	134	Adenovirus C
711	8	AB090341	135	Adenovirus D
712	4	X84646	130	Adenovirus E
714	7	Z48571	129	Types 3, 4, 7 and 21
715	7	Z48571	129	Types 3, 4, 7 and 21
716	7	Z48571	129	Types 3, 4, 7 and 21
717	7	Z48571	129	Types 3, 4, 7 and 21
718	7	Z48571	129	Types 3, 4, 7 and 21
719	7	Z48571	129	Types 3, 4, 7 and 21
720	7	Z48571	129	Types 3, 4, 7 and 21
721	7	Z48571	129	Types 3, 4, 7 and

				21
739	4	X84646	130	Groups A, B, D and E with Type Resolution
740	4	X84646	130	Groups A, B, D and E with Type Resolution
741	4	X84646	130	Type 4 and others
742	4	X84646	130	Type 4 and others
743	4	X84646	130	Type 4 and others
768	4	X84646	130	Groups A, B, D and E with Type Resolution
769	4	X84646	130	Groups A, B, D and E with Type Resolution
901	7	Z48571	129	Types 3, 4, 7 and 21
943	7	Z48571	129	All
944	7	Z48571	129	All
945	7	Z48571	129	All
946	7	Z48571	129	All
947	7	Z48571	129	All
948	7	Z48571	129	All
1113	12	X73487	133	Adenovirus A
1114	12	X73487	133	Adenovirus A
1115	12	X73487	133	Adenovirus A
1116	7	Z48571	129	Adenovirus B
1117	7	Z48571 ---	129	Adenovirus B
1118	1	AF534906	134	Adenovirus C
1119	1	AF534906	134	Adenovirus C
1120	1	AF534906	134	Adenovirus C
1121	8	AB090341	135	Adenovirus D
1122	8	AB090341	135	Adenovirus D
1123	4	X84646	130	Adenovirus E
1124	4	X84646	130	Adenovirus E
1125	4	X84646	130	Adenovirus E

1126	40	L19443	136	Adenovirus F
1127	40	L19443	136	Adenovirus F

Example 3: Sampling Procedures

[210] Samples were gathered from military barracks during an IRB approved study conducted by the Naval Health Research Center Respiratory Disease Laboratory, San Diego. Environmental samples were obtained from eight locations and included surface swabs and air samples collected by dry filter unit air collection and electronic air collectors. Clinical surveillance was conducted by obtaining 1,700 clinical samples from throat, serum and hand swabs using standard clinical protocols which are well known to those with ordinary skill.

Example 4: Sample Preparation and PCR

[211] Samples were processed to obtain viral genomic material using a Qiagen QIAamp Virus BioRobot MDx Kit. Resulting genomic material was amplified using an Eppendorf thermal cycler and the amplicons were characterized on a Bruker Daltonics MicroTOF instrument. The resulting data was analyzed using GenX software (SAIC, San Diego, CA and Ibis, Carlsbad, CA).

[212] All PCR reactions were assembled in 50 µL reaction volumes in a 96-well microtiter plate format using a Packard MPII liquid handling robotic platform and M.J. Dyad thermocyclers (MJ research, Waltham, MA). The PCR reaction mixture consisted of 4 units of AmpliTaq Gold, 1x buffer II (Applied Biosystems, Foster City, CA), 1.5 mM MgCl₂, 0.4 M betaine, 800 µM dNTP mixture and 250 nM of each primer. The following typical PCR conditions were used: 95°C for 10 min followed by 8 cycles of 95°C for 30 seconds, 48°C for 30 seconds, and 72°C 30 seconds with the 48°C annealing temperature increasing 0.9°C with each of the eight cycles. The PCR was then continued for 37 additional cycles of 95°C for 15 seconds, 56°C for 20 seconds, and 72°C 20 seconds.

Example 5: Solution Capture Purification of PCR Products for Mass Spectrometry with Ion Exchange Resin-Magnetic Beads

[213] For solution capture of nucleic acids with ion exchange resin linked to magnetic beads, 25 µl of a 2.5 mg/mL suspension of BioClone amine terminated superparamagnetic beads were added to 25 to 50 µl of a PCR (or RT-PCR) reaction containing approximately 10 pM of a typical PCR amplification product. The above suspension was mixed for approximately 5 minutes by vortexing or pipetting, after which the liquid was removed after using a magnetic separator. The beads containing bound PCR amplification product were then washed three times with 50mM ammonium bicarbonate/50% MeOH or 100mM ammonium bicarbonate/50% MeOH, followed by three more washes with 50% MeOH. The

bound PCR amplicon was eluted with a solution of 25mM piperidine, 25mM imidazole, 35% MeOH which included peptide calibration standards.

Example 6: Mass Spectrometry and Base Composition Analysis

[214] The ESI-FTICR mass spectrometer is based on a Bruker Daltonics (Billerica, MA) Apex II 70e electrospray ionization Fourier transform ion cyclotron resonance mass spectrometer that employs an actively shielded 7 Tesla superconducting magnet. The active shielding constrains the majority of the fringing magnetic field from the superconducting magnet to a relatively small volume. Thus, components that might be adversely affected by stray magnetic fields, such as CRT monitors, robotic components, and other electronics, can operate in close proximity to the FTICR spectrometer. All aspects of pulse sequence control and data acquisition were performed on a 600 MHz Pentium II data station running Bruker's Xmass software under Windows NT 4.0 operating system. Sample aliquots, typically 15 µl, were extracted directly from 96-well microtiter plates using a CTC HTS PAL autosampler (LEAP Technologies, Carrboro, NC) triggered by the FTICR data station. Samples were injected directly into a 10 µl sample loop integrated with a fluidics handling system that supplies the 100 µl /hr flow rate to the ESI source. Ions were formed via electrospray ionization in a modified Analytica (Branford, CT) source employing an off axis, grounded electrospray probe positioned approximately 1.5 cm from the metallized terminus of a glass desolvation capillary. The atmospheric pressure end of the glass capillary was biased at 6000 V relative to the ESI needle during data acquisition. A counter-current flow of dry N₂ was employed to assist in the desolvation process. Ions were accumulated in an external ion reservoir comprised of an rf-only hexapole, a skimmer cone, and an auxiliary gate electrode, prior to injection into the trapped ion cell where they were mass analyzed. Ionization duty cycles greater than 99% were achieved by simultaneously accumulating ions in the external ion reservoir during ion detection. Each detection event consisted of 1M data points digitized over 2.3 s. To improve the signal-to-noise ratio (S/N), 32 scans were co-added for a total data acquisition time of 74 s.

[215] The ESI-TOF mass spectrometer is based on a Bruker Daltonics MicroTOF™. Ions from the ESI source undergo orthogonal ion extraction and are focused in a reflectron prior to detection. The TOF and FTICR are equipped with the same automated sample handling and fluidics described above. Ions are formed in the standard MicroTOF™ ESI source that is equipped with the same off-axis sprayer and glass capillary as the FTICR ESI source. Consequently, source conditions were the same as those described above. External ion accumulation was also employed to improve ionization duty cycle during data acquisition. Each detection event on the TOF was comprised of 75,000 data points digitized over 75 µs.

[216] The sample delivery scheme allows sample aliquots to be rapidly injected into the electrospray source at high flow rate and subsequently be electrosprayed at a much lower flow rate for improved ESI

sensitivity. Prior to injecting a sample, a bolus of buffer was injected at a high flow rate to rinse the transfer line and spray needle to avoid sample contamination/carryover. Following the rinse step, the autosampler injected the next sample and the flow rate was switched to low flow. Following a brief equilibration delay, data acquisition commenced. As spectra were co-added, the autosampler continued rinsing the syringe and picking up buffer to rinse the injector and sample transfer line. In general, two syringe rinses and one injector rinse were required to minimize sample carryover. During a routine screening protocol a new sample mixture was injected every 106 seconds. More recently a fast wash station for the syringe needle has been implemented which, when combined with shorter acquisition times, facilitates the acquisition of mass spectra at a rate of just under one spectrum/minute.

[217] Raw mass spectra were post-calibrated with an internal mass standard and deconvoluted to monoisotopic molecular masses. Unambiguous base compositions were derived from the exact mass measurements of the complementary single-stranded oligonucleotides. Quantitative results are obtained by comparing the peak heights with an internal PCR calibration standard present in every PCR well at 500 molecules per well. Calibration methods are commonly owned and disclosed in U.S. Provisional Patent Application Serial No. 60/545,425 which is incorporated herein by reference in entirety.

Example 7: De Novo Determination of Base Composition of Amplification Products using Molecular Mass Modified Deoxynucleotide Triphosphates

[218] Because the molecular masses of the four natural nucleobases have a relatively narrow molecular mass range ($A = 313.058$, $G = 329.052$, $C = 289.046$, $T = 304.046$ – See Table 4), a persistent source of ambiguity in assignment of base composition can occur as follows: two nucleic acid strands having different base composition may have a difference of about 1 Da when the base composition difference between the two strands is $G \leftrightarrow A$ (-15.994) combined with $C \leftrightarrow T$ (+15.000). For example, one 99-mer nucleic acid strand having a base composition of $A_{27}G_{30}C_{21}T_{21}$ has a theoretical molecular mass of 30779.058 while another 99-mer nucleic acid strand having a base composition of $A_{26}G_{31}C_{22}T_{20}$ has a theoretical molecular mass of 30780.052. A 1 Da difference in molecular mass may be within the experimental error of a molecular mass measurement and thus, the relatively narrow molecular mass range of the four natural nucleobases imposes an uncertainty factor.

[219] The present invention provides for a means for removing this theoretical 1 Da uncertainty factor through amplification of a nucleic acid with one mass-tagged nucleobase and three natural nucleobases. The term “nucleobase” as used herein is synonymous with other terms in use in the art including “nucleotide,” “deoxynucleotide,” “nucleotide residue,” “deoxynucleotide residue,” “nucleotide triphosphate (NTP),” or deoxynucleotide triphosphate (dNTP).

[220] Addition of significant mass to one of the 4 nucleobases (dNTPs) in an amplification reaction, or in the primers themselves, will result in a significant difference in mass of the resulting amplification product (significantly greater than 1 Da) arising from ambiguities arising from the G ↔ A combined with C ↔ T event (Table 4). Thus, the same the G ↔ A (-15.994) event combined with 5-Iodo-C ↔ T (-110.900) event would result in a molecular mass difference of 126.894. If the molecular mass of the base composition A₂₇G₃₀**5-Iodo-C₂₁T₂₁** (33422.958) is compared with A₂₆G₃₁**5-Iodo-C₂₂T₂₀**, (33549.852) the theoretical molecular mass difference is +126.894. The experimental error of a molecular mass measurement is not significant with regard to this molecular mass difference. Furthermore, the only base composition consistent with a measured molecular mass of the 99-mer nucleic acid is A₂₇G₃₀**5-Iodo-C₂₁T₂₁**. In contrast, the analogous amplification without the mass tag has 18 possible base compositions.

Table 4: Molecular Masses of Natural Nucleobases and the Mass-Modified Nucleobase 5-Iodo-C and Molecular Mass Differences Resulting from Transitions

Nucleobase	Molecular Mass	Transition	Molecular Mass
A	313.058	A-->T	-9.012
A	313.058	A-->C	-24.012
A	313.058	A--> 5-Iodo-C	101.888
A	313.058	A-->G	15.994
T	304.046	T-->A	9.012
T	304.046	T-->C	-15.000
T	304.046	T--> 5-Iodo-C	110.900
T	304.046	T-->G	25.006
C	289.046	C-->A	24.012
C	289.046	C-->T	15.000
C	289.046	C-->G	40.006
5-Iodo-C	414.946	5-Iodo-C-->A	-101.888
5-Iodo-C	414.946	5-Iodo-C-->T	-110.900
5-Iodo-C	414.946	5-Iodo-C-->G	-85.894
G	329.052	G-->A	-15.994
G	329.052	G-->T	-25.006
G	329.052	G-->C	-40.006
G	329.052	G--> 5-Iodo-C	85.894

[221] Mass spectra of bioagent-identifying amplicons were analyzed independently using a maximum-likelihood processor, such as is widely used in radar signal processing. This processor, referred to as GenX, first makes maximum likelihood estimates of the input to the mass spectrometer for each primer by running matched filters for each base composition aggregate on the input data. This includes the GenX response to a calibrant for each primer.

[222] The algorithm emphasizes performance predictions culminating in probability-of-detection versus probability-of-false-alarm plots for conditions involving complex backgrounds of naturally occurring organisms and environmental contaminants. Matched filters consist of *a priori* expectations of signal values given the set of primers used for each of the bioagents. A genomic sequence database is used to define the mass base count matched filters. The database contains the sequences of known bacterial bioagents and includes threat organisms as well as benign background organisms. The latter is used to estimate and subtract the spectral signature produced by the background organisms. A maximum likelihood detection of known background organisms is implemented using matched filters and a running-sum estimate of the noise covariance. Background signal strengths are estimated and used along with the matched filters to form signatures which are then subtracted. The maximum likelihood process is applied to this "cleaned up" data in a similar manner employing matched filters for the organisms and a running-sum estimate of the noise-covariance for the cleaned up data.

[223] The amplitudes of all base compositions of bioagent-identifying amplicons for each primer are calibrated and a final maximum likelihood amplitude estimate per organism is made based upon the multiple single primer estimates. Models of all system noise are factored into this two-stage maximum likelihood calculation. The processor reports the number of molecules of each base composition contained in the spectra. The quantity of amplification product corresponding to the appropriate primer set is reported as well as the quantities of primers remaining upon completion of the amplification reaction.

[224] Base count blurring can be carried out as follows. "Electronic PCR" can be conducted on nucleotide sequences of the desired bioagents to obtain the different expected base counts that could be obtained for each primer pair. See for example, ncbi.nlm.nih.gov/sutils/e-pcr/; Schuler, *Genome Res.* 7:541-50, 1997. In one illustrative embodiment, one or more spreadsheets, such as Microsoft Excel workbooks contain a plurality of worksheets. First in this example, there is a worksheet with a name similar to the workbook name; this worksheet contains the raw electronic PCR data. Second, there is a worksheet named "filtered bioagents base count" that contains bioagent name and base count; there is a separate record for each strain after removing sequences that are not identified with a genus and species and removing all sequences for bioagents with less than 10 strains. Third, there is a worksheet, "Sheet1" that contains the frequency of substitutions, insertions, or deletions for this primer pair. This data is generated by first creating a pivot table from the data in the "filtered bioagents base count" worksheet and then executing an Excel VBA macro. The macro creates a table of differences in base counts for bioagents of the same species, but different strains. One of ordinary skill in the art may understand additional pathways for obtaining similar table differences without undo experimentation.

[225] Application of an exemplary script, involves the user defining a threshold that specifies the fraction of the strains that are represented by the reference set of base counts for each bioagent. The reference set of base counts for each bioagent may contain as many different base counts as are needed to meet or exceed the threshold. The set of reference base counts is defined by taking the most abundant strain's base type composition and adding it to the reference set and then the next most abundant strain's base type composition is added until the threshold is met or exceeded. The current set of data was obtained using a threshold of 55%, which was obtained empirically.

[226] For each base count not included in the reference base count set for that bioagent, the script then proceeds to determine the manner in which the current base count differs from each of the base counts in the reference set. This difference may be represented as a combination of substitutions, $Si=Xi$, and insertions, $Ii=Yi$, or deletions, $Di=Zi$. If there is more than one reference base count, then the reported difference is chosen using rules that aim to minimize the number of changes and, in instances with the same number of changes, minimize the number of insertions or deletions. Therefore, the primary rule is to identify the difference with the minimum sum ($Xi+Yi$) or ($Xi+Zi$), e.g., one insertion rather than two substitutions. If there are two or more differences with the minimum sum, then the one that will be reported is the one that contains the most substitutions.

[227] Differences between a base count and a reference composition are categorized as one, two, or more substitutions, one, two, or more insertions, one, two, or more deletions, and combinations of substitutions and insertions or deletions. The different classes of nucleobase changes and their probabilities of occurrence have been delineated in U.S. Patent Application Publication No. 2004209260 (U.S. Application Serial No. 10/418,514) which is incorporated herein by reference in entirety.

Example 8: Identification of Adenoviruses

[228] The purpose of this series of experiments was to investigate the spread of adenovirus within a military installation by establishing a temporal relationship between the environmental presence of adenovirus and resulting illness in military personnel, as well as evaluation of asymptomatic carriage. In the military installation, adenovirus has been determined to be the cause of 72% of respiratory illness during the winter. Adenovirus is known to spread rapidly among recruits at the military installation, with outbreaks yielding 50 to 80% attack rates.

[229] Primer pair nos. 615 (SEQ ID NOs: 45:102) and 616 (46:100) were tested in quadruplicate against representative human adenovirus species. Both primer pairs gave rise to amplification products for adenovirus types 4, 7, 8 and 40 from which high quality mass spectral signals were obtained. Adenovirus

type 12 was also observed but the mass spectral signals were not as strong. Adenovirus type 1 was observed with weak mass spectral signals. Base compositions were determined from the molecular masses of the amplification products and were found to be in agreement with the base compositions calculated for the bioagent identifying amplicons of adenovirus types 4, 7, 8 and 40 defined by the primer pairs.

[230] Primer pair number 739 (SEQ ID NOS: 30:101), a general survey primer, was found to produce primer dimers indicated by agarose gel electrophoresis. This primer pair was redesigned and tested. The best redesigned primer pair is primer pair number 769 (SEQ ID NOS: 26:121).

[231] Shown in Figure 4 are mass spectra of amplification products corresponding to adenoviral bioagent identifying amplicons obtained by amplification of samples with primer pair number 943 (SEQ ID NOS: 61:122) according to procedures outlined in Example 3 followed by purification according to Example 4 and analysis of base composition according to examples 5 and 6. It is seen that the single primer pair produced adenoviral bioagent identifying amplicons whose molecular masses can be deconvolved to distinct base compositions for adenovirus types 21, 12, 8, 7, and 4. Thus, each of these adenovirus types can be efficiently distinguished from each other.

[232] A calibration sequence based on the bioagent identifying amplicon produced by primer pair number 943 and reference sequence of adenovirus serotype 4 (GenBank accession no: X84646) was tested for the ability to quantify known amounts of adenovirus serotype 4. It was determined that adenovirus serotype 4 could be detected at levels as low as 15-30 genomes per sample using primer pair number 943 (SEQ ID NOS: 61:122). A representative mass spectrum of amplification products corresponding to adenovirus identifying amplicons and calibration amplicons obtained with primer pair number 943 (SEQ ID NOS: 61:122) is shown in Figure 5.

[233] The limits of detection of adenoviruses in throat swabs for the two primer set comprising primer pair numbers 769 (SEQ ID NOS: 26:121) and 943 (SEQ ID NOS: 61:122) were found to be 15-30 genome copies per sample. Limits of detection in air background and in no background (clean sample) were found to be 30 genome copies per sample.

[234] In another experiment, the ability to identify diverse adenovirus types with primer pair numbers 769 (SEQ ID NOS: 26:121) and 943 (SEQ ID NOS: 61:122) was evaluated by spiking different adenovirus types representing different adenovirus subgroups into a sample and analyzing the sample by obtaining

amplification products corresponding to bioagent identifying amplicons of the adenovirus nucleic acid with the primers and analyzing the amplification products by mass spectrometry. The base compositions of the amplification products were calculated from the molecular masses and used to make the adenovirus type assignments. The results are shown in Table 5.

Table 5: Identification of Adenoviruses From Amplification Products Obtained With Primer Pair Numbers 769 and 943

Sample Number	Primer Pair	Adenovirus Type Spiked Into Sample	Adenovirus Spike Subgroup	Adenovirus Type Identified	Base Composition Result	Adenovirus Subgroup Identified
1	943	4	E	4	A20G34C38T20	E
1	769	4	E	4	A27G33C39T22	E
2	943	3	B	Type 3 with G->A SNP	A23G31C37T21	B
2	769	3	B	3	A27G37C24T33	B
3	943	40	F	40	A21G33C39T19	F
3	769	40	F	NA-No Product Expected	NA	NA
4	943	13	D	17, 48	A18G38C36T20	D
4	769	13	D	37	A28G28C44T21	D
5	943	Mouse	Murine A	Human type 4, Simian type 22, Simian type 25	A20G34C38T20	E
5	769	Mouse	Murine A	Murine Adeno 1	A37G25C33T26	A
6	943	6	C	1, 2, 5, 4	A20G33C39T20	C, E
6	769	6	C	NA	NA	NA
7	943	10	D	17, 48	A20G36C38T18	D
7	769	10	D	9	A28G29C44T20	D
8	943	31	A	Type 12 with a T->C SNP	A20G32C38T22	A
8	769	31	A	NA-No Product Expected	NA	NA
9	943	18	A	Closest Match Type 12	A21G32C36T23	A
9	769	18	A	Closest Match is Bovine Type A	A31G29C31T30	NA
10	943	Simian	C1	Simian type 21, Human 21, 34	A21G33C37T21	B
10	769	Simian	C1	NA-Not Known If Priming Expected	NA	NA

[235] In another experiment, testing of air samples containing spikes of adenovirus was performed. A total of 35 spiked dry filter unit air samples were provided by a military installation. The adenovirus type 4 spike concentration levels (in plate-forming units – PFU) varied between 5.62×10^5 to 5.62 PFU in the presence and absence of Triton-X100 detergent on the filter surface. Sample collections from the dry filter unit were taken over a period of 12 hours and were analyzed by obtaining amplification products with primer pair numbers 769 (SEQ ID NOS: 26:121) and 943 (SEQ ID NOS: 61:122), and analyzing the products by mass spectrometry. Adenovirus was identified at concentrations as low as 5.62 PFU with no sensitivity to the presence of the detergent and no difference in identification of adenovirus over the 12 hour period.

[236] In another experiment, environmental and clinical surveillance was undertaken within a military installation. A total of 1,600 environmental samples including surface swabs and dry filter unit air samples were taken from various locations within the barracks. A total of 1,700 clinical samples including throat, serum and hand swabs were obtained using standard protocols from symptomatic and asymptomatic military recruits occupying the barracks. All samples were tested for the presence of adenovirus by the method of the present invention. Cultures were grown for 785 of the clinical samples. The results of positive and negative identification of adenovirus in this 785 sample subgroup are shown in Table 6. These results indicate that the method of the present invention is more sensitive for identification of the presence of adenovirus than the standard culture method. In all cases, adenovirus Type 4 was identified. This provides an indication that adenovirus Type 4 is indigenous to the military barracks from which the samples were obtained and also indicates that the method of the present invention is particularly useful for epidemiological investigations of the spread of pathogens in individuals and in the environment.

Table 6: Comparison of the Present Invention with Standard Culture Methods for Identification of

Adenovirus

Test Result by Present Invention	Test Result by Standard Culture Method	Sample Numbers
Positive	Positive	135
Negative	Positive	0
Positive	Negative	78
Negative	Negative	572

[237] The present invention includes any combination of the various species and subgeneric groupings falling within the generic disclosure. This invention therefore includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[238] While in accordance with the patent statutes, description of the various embodiments and examples have been provided, the scope of the invention is not to be limited thereto or thereby. Modifications and alterations of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention.

[239] Therefore, it will be appreciated that the scope of this invention is to be defined by the appended claims, rather than by the specific examples which have been presented by way of example.

[240] Each reference (including, but not limited to, journal articles, U.S. and non-U.S. patents, patent application publications, international patent application publications, gene bank accession numbers, internet web sites, and the like) cited in the present application is incorporated herein by reference in its entirety.

CLAIMS

What is claimed is:

1. An oligonucleotide primer 14 to 35 nucleobases in length comprising at least 70% sequence identity with SEQ ID NO: 26.
2. An oligonucleotide primer 14 to 35 nucleobases in length comprising at least 70% sequence identity with SEQ ID NO: 121.
3. A composition comprising the primer of claim 1.
4. The composition of claim 3 further comprising an oligonucleotide primer 14 to 35 nucleobases in length comprising at least 70% sequence identity with SEQ ID NO: 121.
5. The composition of claim 4 wherein either or both of said primers comprises at least one modified nucleobase.
6. The composition of claim 5 wherein said modified nucleobase is 5-propynyluracil or 5-propynylcytosine.
7. The composition of claim 4 wherein either or both of said primers comprises at least one universal nucleobase.
8. The composition of claim 7 wherein said universal nucleobase is inosine.
9. The composition of claim 4 wherein either or both of said primers further comprises a non-templated T residue on the 5'-end.
10. The composition of claim 4 wherein either or both of said primers comprises at least one non-template tag.
11. The composition of claim 4 wherein either or both of said primers comprises at least one molecular mass modifying tag.
12. A kit comprising the composition of claim 4.

13. The kit of claim 12 further comprising one or more primer pairs wherein each member of said one or more primer pairs is of a length of 14 to 35 nucleobases and has 70% to 100% sequence identity with the corresponding member from the group of primer pairs represented by SEQ ID NOs: 61:122, 38:82, 36:95, 19:93, 54:113, 36:98 and 16:106.

14. The kit of claim 12 further comprising at least one calibration polynucleotide.

15. The kit of claim 12 further comprising at least one anion exchange functional group linked to a magnetic bead.

16. A method for identification of an adenovirus in a sample comprising:

amplifying nucleic acid from said adenovirus using the composition of claim 4 to obtain an amplification product;

determining the molecular mass of said amplification product;

optionally, determining the base composition of said amplification product from said molecular mass; and

comparing said molecular mass or base composition with a plurality of molecular masses or base compositions of known adenovirus identifying amplicons, wherein a match between said molecular mass or base composition and a member of said plurality of molecular masses or base compositions identifies said adenovirus.

17. The method of claim 16 wherein said sample is a biological product.

18. A method of determining the presence or absence of a adenovirus in a sample comprising:

amplifying nucleic acid from said sample using the composition of claim 4 to obtain an amplification product;

determining the molecular mass of said amplification product;

optionally, determining the base composition of said amplification product from said molecular mass; and

comparing said molecular mass or base composition of said amplification product with the known molecular masses or base compositions of one or more known adenovirus identifying amplicons, wherein a match between said molecular mass or base composition of said amplification product and the molecular mass or base composition of one or more known adenovirus identifying amplicons indicates the presence of said adenovirus in said sample.

19. The method of claim 18 wherein said sample comprises a biological product.

20. A method for determination of the quantity of an unknown adenovirus in a sample comprising:
contacting said sample with the composition of claim 4 and a known quantity of a calibration polynucleotide comprising a calibration sequence;

concurrently amplifying nucleic acid from said unknown adenovirus and nucleic acid from said calibration polynucleotide in said sample with the composition of claim 4 to obtain a first amplification product comprising a adenovirus identifying amplicon and a second amplification product comprising a calibration amplicon;

determining the molecular mass and abundance for said adenovirus identifying amplicon and said calibration amplicon; and

distinguishing said adenovirus identifying amplicon from said calibration amplicon based on molecular mass, wherein comparison of adenovirus identifying amplicon abundance and calibration amplicon abundance indicates the quantity of adenovirus in said sample.

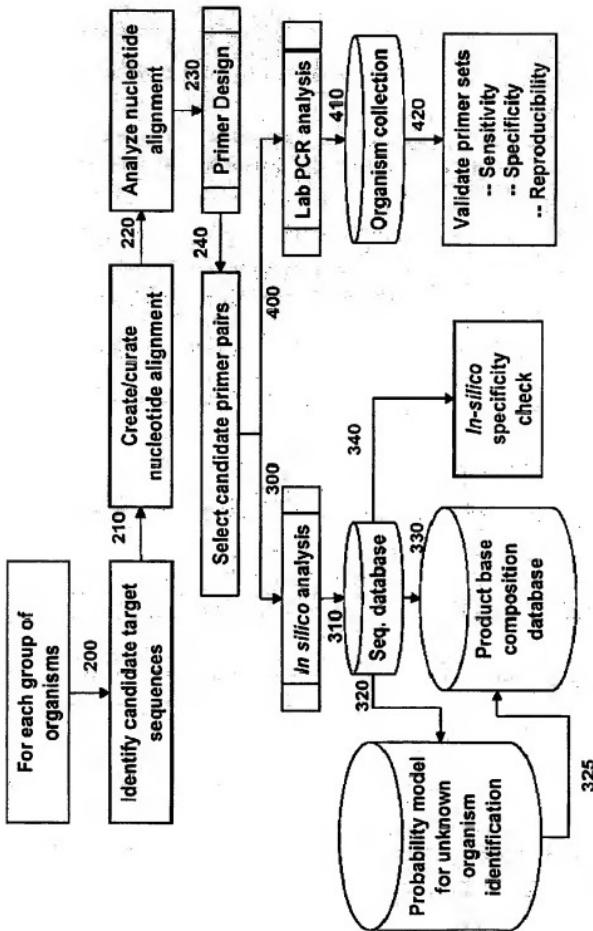


Figure 1

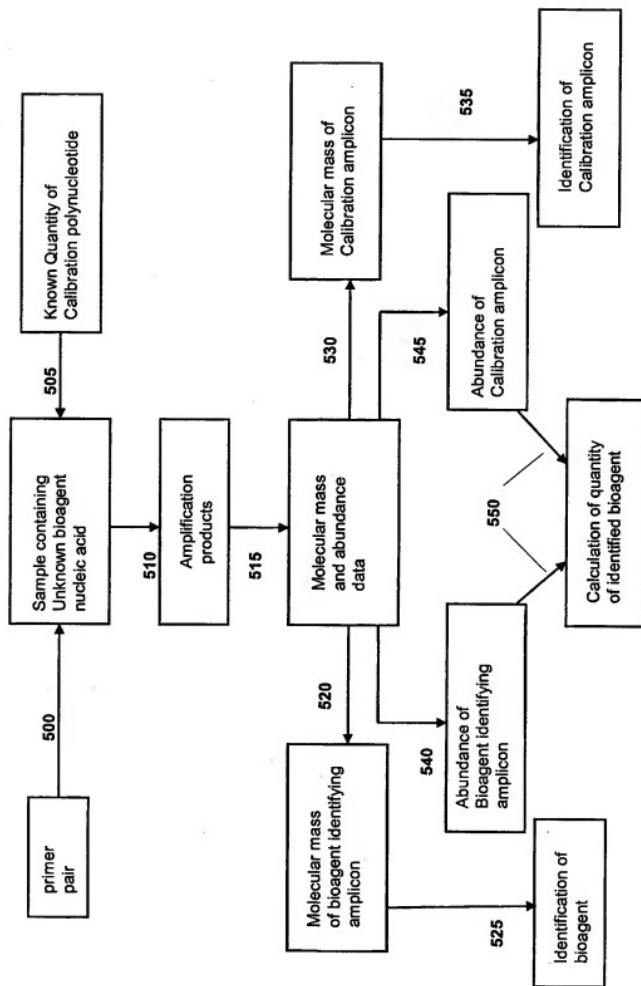


Figure 2

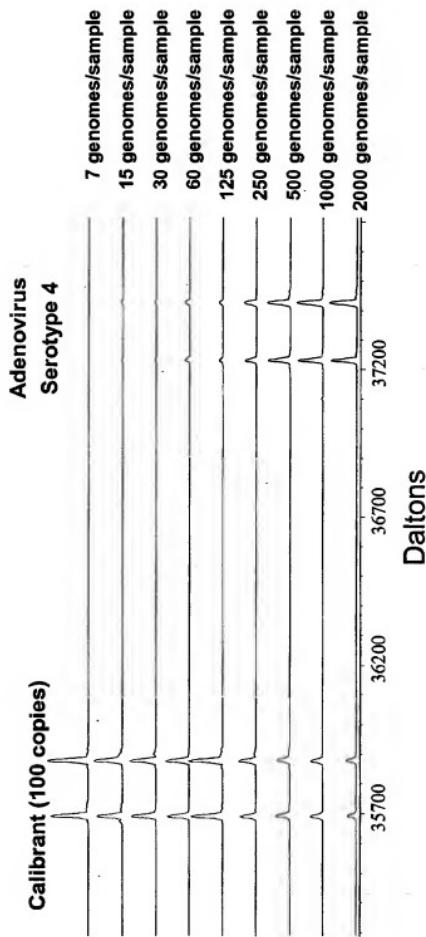


Figure 3

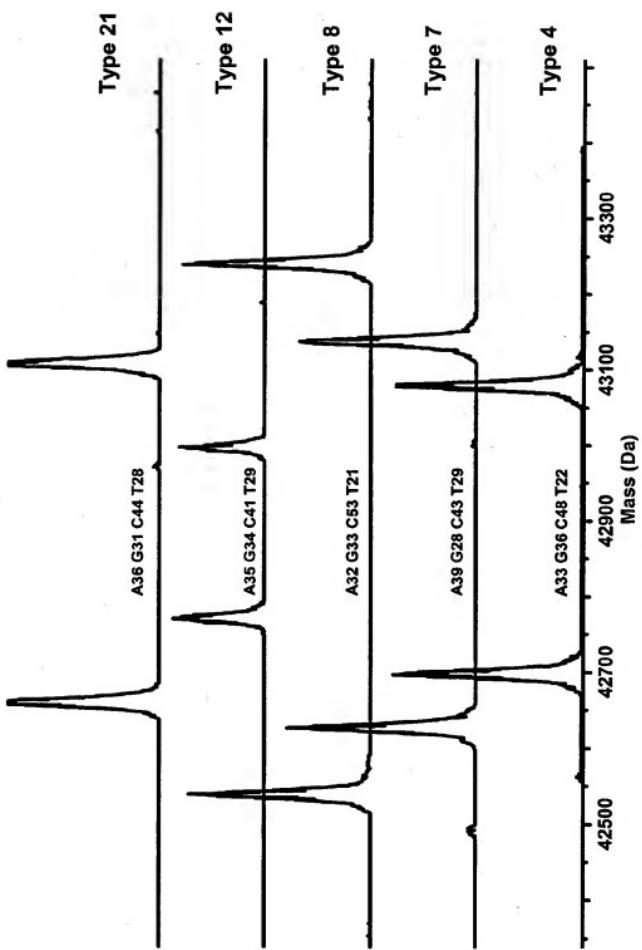


Figure 4

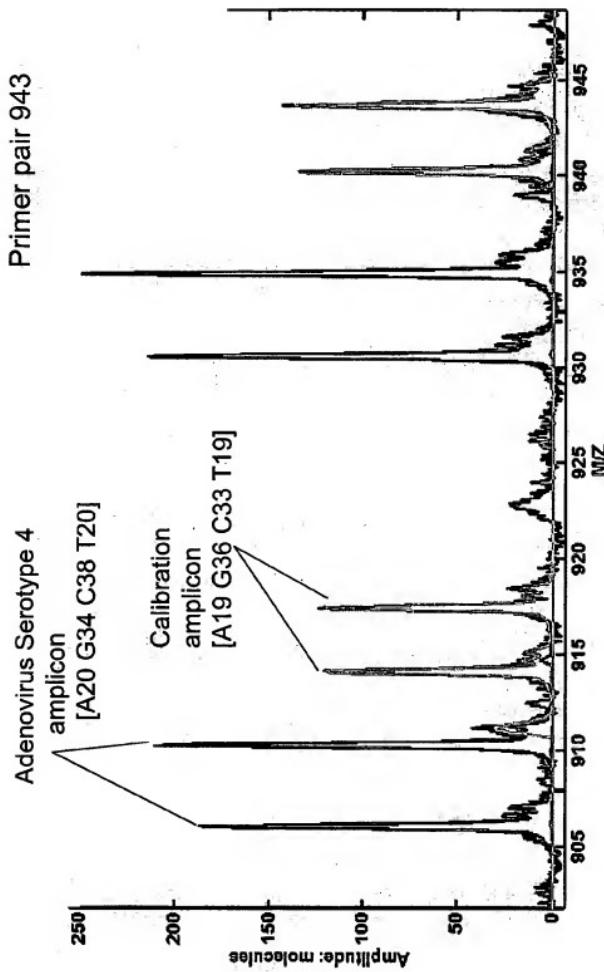


Figure 5

SEQUENCE LISTING

<110> Hall, Thomas A.
Sampath, Rangarajan
Blyn, Lawrence

<120> COMPOSITIONS FOR USE IN IDENTIFICATION
OF ADENOVIRUSES

<130> DIBIS-0073WO

<150> 60/671,003

<151> 2005-04-13

<160> 136

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 1

acagacactt accagggtg

19

<210> 2

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 2

agacccaatt acattggctt

20

<210> 3

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 3

agtccgggtc tggtgca

18

<210> 4

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 4

atgccaccc catcgatg

18

<210> 5

<211> 20

<212> DNA

<213> Artificial Sequence

<220>
<223> Primer

<400> 5
atgtactaca acagtaactgg 20

<210> 6
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 6
caatccgttc tggttccgga tgaa 24

<210> 7
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 7
cggatccaag ctaatctttg g 21

<210> 8
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 8
gatatggcca ccccatcgat 20

<210> 9
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 9
ggaaagacat tactgcagac a 21

<210> 10
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 10
ggaatttttt gatggtagag a 21

<210> 11
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 11	
gggcattatgt actacaacag	20
<210> 12	
<211> 23	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 12	
ggtcgtttagt tgcccttcca cat	23
<210> 13	
<211> 30	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 13	
taaaaaggta tcaatcatgt ttgactcctc	30
<210> 14	
<211> 24	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 14	
taaacacctac gagttacatga acgg	24
<210> 15	
<211> 24	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 15	
taacagaccc aattacattt gctt	24
<210> 16	
<211> 17	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 16	
taaagcgcccg ataccctt	17
<210> 17	
<211> 20	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 17	

tacagacact taccagggtg	20
<210> 18	
<211> 21	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 18	
taccttcaa cctgaacctc a	21
<210> 19	
<211> 26	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 19	
tagactaca tgaacaagcg agtgt	26
<210> 20	
<211> 25	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 20	
tactacaaca gcactggcaa tatgg	25
<210> 21	
<211> 27	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 21	
tagaggaaaa atatggaggc agagctc	27
<210> 22	
<211> 20	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 22	
tagatctggc tttctttgac	20
<210> 23	
<211> 19	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 23	
tcaacatggg tgtgctggc	19

<210> 24
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 24
tcaatgggca tacatgcaca tc 22

<210> 25
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 25
tcaccaaac ac ctacgagtac atga 24

<210> 26
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 26
tcaccaaac ac ctacgagtac atgaa 25

<210> 27
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 27
tcactaaaga caaaggcttt cc 22

<210> 28
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 28
tcagccagag ccgcaagtag 20

<210> 29
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 29
tcatgtctacg ggtctttgc 20

<210> 30
<211> 29

<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 30
tccaaacacca acacctacga gtacatgaa 29

<210> 31
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 31
tcccaatggg catacatgca catc 24

<210> 32
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 32
tccttacttt gtatactctg gaaccattcc 30

<210> 33
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 33
tcctaaatac tggatgg 27

<210> 34
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 34
tcctaaatac tggatgg 27

<210> 35
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 35
tcctgttgag aaatttcctg tactccaa 28

<210> 36
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 36
tcgccaagcc taccaacaaa gaagg 25

<210> 37
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 37
tctacacctgt cgctgcaaac atg 23

<210> 38
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 38
tctttatcgc cagagccgca agtag 25

<210> 39
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 39
tctttatgtac tacaacagca ctggaa 25

<210> 40
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 40
tgaatgctgt ggttgacttg caaga 25

<210> 41
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 41
tgaatgctgt ggttgacttg caagacaga 29

<210> 42
<211> 19
<212> DNA
<213> Artificial Sequence

<220>

<223> Primer

<400> 42

tgacagacac ttaccaggg

19

<210> 43

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 43

tgatatacat ctggcttct ttgac

25

<210> 44

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 44

tgatatcaact aaagacaaaag gtcttcc

27

<210> 45

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 45

tgatatggcc accccatcga t

21

<210> 46

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 46

tgatatggcc accccatcga tg

22

<210> 47

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 47

tgcagaatgg ccacccatc gatg

24

<210> 48

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 48	
tgc ccatgcta cgggtctttt gc	22
<210> 49	
<211> 26	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 49	
tgc cccaatg ggcatacatg cacatc	26
<210> 50	
<211> 25	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 50	
tgc ttatgtta ctacaacagc actgg	25
<210> 51	
<211> 25	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 51	
tgc ttatgtta ctacaacagc actgg	25
<210> 52	
<211> 28	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 52	
tgg aacttca ggaaggatgt taacatgg	28
<210> 53	
<211> 22	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 53	
tgg aattttt cgatggtaga ga	22
<210> 54	
<211> 21	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 54	
tgg caacatg ggtgtgctgg c	21

<210> 55
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 55
tgggattgac agatacttac caggg 25

<210> 56
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 56
tggtaaaatc ctgttatggt tcattcgc 28

<210> 57
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 57
tgtcggtt atgtactaca acag 24

<210> 58
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 58
tgtgccttcc cacatacagg tgcc 24

<210> 59
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 59
ttatgatata gagctggctt tcttgaca 29

<210> 60
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 60
ttccatgccc aacagaccca actaca 26

<210> 61

<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 61
ttgcaagatg gccaccccat cgat

24

<210> 62
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 62
ttgaaagac attactgcag aca

23

<210> 63
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 63
tttcaagtgc ctcagaaatt ctttgctg

28

<210> 64
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 64
atgtggtgt catctttgtc

20

<210> 65
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 65
atatgatgtat ctggagtctg c

21

<210> 66
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 66
caagtcaacc acagcattca

20

<210> 67
<211> 20
<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 67

ccaaaccttag gctctggctg

20

<210> 68

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 68

ccagtgcgtgt tgttagtacat

20

<210> 69

<211> 17

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 69

cgttcgggtgg tcacatc

17

<210> 70

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 70

ccagtgcgtgt tgttagtacat

20

<210> 71

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 71

ctgtccggcg atgtgcattg

19

<210> 72

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 72

cttgcgggtc gttcaaagag gtag

24

<210> 73

<211> 21

<212> DNA

<213> Artificial Sequence

<220>
<223> Primer
<400> 73
ggcttcggcg tctgttaattt c 21
<210> 74
<211> 18
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 74
gggcgaactg caccagac 18
<210> 75
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 75
ggtatgtact catagggtt ggtg 24
<210> 76
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 76
taaagcacaa ttccaggcg 19
<210> 77
<211> 30
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 77
taagtgaaca ttttctgcgt acattacaat 30
<210> 78
<211> 29
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 78
taagtgaaca ttttctgcta cattacaat 29
<210> 79
<211> 28
<212> DNA
<213> Artificial sequence

<220>
<223> Primer 13

<400> 79
tactatcaa gaaaggcagg tcttatatc 28

<210> 80
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 80
tac~~tgttgtg~~ tcattttg c 21

<210> 81
<211> 24
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 81
tagaccgg a ctcaggtact ccga 24

<210> 82
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 82
tagcgttagg a gccatagcac g 21

<210> 83
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 83
tagctgtcca cagcctgatt ccaca 25

<210> 84
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 84
tagctgtcca ccgcctgatt ccaca 25

<210> 85
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 85

taggaccatg ttcacatcct tgctgaagtt cca	33
<210> 86	
<211> 20	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 86	
taggcggtgt tggtggccat	20
<210> 87	
<211> 22	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 87	
tatatgagta tctggagtct gc	22
<210> 88	
<211> 20	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 88	
tcaactttag gctctggctg	20
<210> 89	
<211> 30	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 89	
tcaactttagt gcctatggct atttcattag	30
<210> 90	
<211> 26	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 90	
tcacccctctt tgggtttagg cttggc	26
<210> 91	
<211> 27	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 91	
tcagcccaat ttgcgcagg aatagaa	27

<210> 92
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 92
tccacccatc aaaaaattcc atgtcaatat c 31

<210> 93
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 93
tcacggatcg cggtgttgtt t 21

<210> 94
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 94
tccatcgaaa aattccatgt caatatc 27

<210> 95
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 95
tccatgttca catcctttct gaagttcca 29

<210> 96
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 96
tccatcgaaa aattccatgt caatatc 27

<210> 97
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 97
tccccatcca cagaacgcctt tatttcaa 28

<210> 98
<211> 28

<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 98
tcctgtttt tgctgtcaaa gaaagcca 28

<210> 99
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 99
tcctttctga agttccactc atagg 25

<210> 100
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 100
tcgcgggcga actgca 16

<210> 101
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 101
tcgcgttgcg gtgggtgtt 19

<210> 102
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 102
tcggggcgaac tgcaccag 18

<210> 103
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 103
tcgggtatcc tgtcttgcaa gtc 23

<210> 104
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 104
tctgtcttgc aagtcaacca c 21

<210> 105
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 105
tgAACCGTAG CATGGTTCA T 21

<210> 106
<211> 25
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 106
tgAAgTTGTC CCTAAAACCA ATGTA 25

<210> 107
<211> 23
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 107
tgACCAGCCA GCACACCCAT GTT 23

<210> 108
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 108
tgATTCCAT GGCAAAGGA TT 22

<210> 109
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Primer

<400> 109
tgATTCCAT GGCAAAGGA TT 22

<210> 110
<211> 22
<212> DNA
<213> Artificial sequence

<220>

<223> Primer

<400> 110
tgcaagtcaa ccacagcatt ca

22

<210> 111

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 111
tgccagtgtc gttttagtac at

22

<210> 112

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 112
tgcgttaggag ccatagcacg

20

<210> 113

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 113
tgctcggtat ttctgtcttg caagtc

26

<210> 114

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 114
tgcttcgccc tctgttaattt c

21

<210> 115

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 115
tggaggatc aaacatgatt gacacct

27

<210> 116

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Primer

<400> 116	
tggcgcgggc gaactgca	18
<210> 117	
<211> 19	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 117	
tgggagccac cactcgctt	19
<210> 118	
<211> 17	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 118	
tggcgaact gcaccagg	17
<210> 119	
<211> 19	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 119	
tggcgaact gcaccagac	19
<210> 120	
<211> 25	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 120	
tgtttgaagg gatttacgtt gtccca	25
<210> 121	
<211> 26	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 121	
tgtttgaagg gatttacgtt gtcccat	26
<210> 122	
<211> 20	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 122	
tgtggcgcggc gcgaaactgca	20

<210> 123	
<211> 20	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 123	
tgttgcgcgg gcgaactgca	20
<210> 124	
<211> 25	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 124	
tgtttctgtc ttgcaagtca accac	25
<210> 125	
<211> 22	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 125	
tgcagaatcaa ccacacgatt ca	22
<210> 126	
<211> 20	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 126	
ttaaaggcaca atttcaggcg	20
<210> 127	
<211> 20	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 127	
ttgcgggtgt ggttgaaggg	20
<210> 128	
<211> 26	
<212> DNA	
<213> Artificial Sequence	
<220>	
<223> Primer	
<400> 128	
ttgttcacat ctttgctgaa gttcca	26
<210> 129	

<211> 2918

<212> DNA

<213> Adenovirus

<220>

<400> 129

tcatcaccc	gcccggcag	cagaggagaa	agaagagggt	Cgcgcgcca	ggctgagttg	60
cttcaagat	ggccacccca	tcgatgaigc	cccaatgggc	atacatgcac	atcgccggac	120
aggatgttc	ggatgtactg	agtccgggtc	tggtgcaagg	cgccccgtca	acagacact	180
acttcgat	ggggacaaaa	tttagaaacc	ccacagtgg	gcccacccat	gatgtgacca	240
ccgacccgt	caagcactg	atgtcgct	tgatcccggt	tgacccggaa	gacaatacc	300
actttcaaa	actgtcgctac	acgtcggtc	taggcgacaa	cagagtgcct	gacatggcc	360
gcacattctt	tgacattcgg	ggggtgtctt	ataagggtcc	tagcttaag	ccatatictg	420
gcacagctt	caattcactg	gttcctaagg	gcgcggccaa	tacatctcg	tgatagttt	480
caggcaggaa	agaadagcga	gtaaatccca	ccacaacac	atttgcatt	gcttcatgt	540
aggggacaaa	tattttaaa	aaagggttag	aatttggaa	agacattact	cgacacaaac	600
agccatctt	tgccqataaa	acatcatcg	cagacccgt	agtggagaa	gaatctatgg	660
ctgtatctgt	tgaaacaaaat	aaaaatgtt	gagggttagc	tcttaaccac	gtacccaaa	720
tgaqcctatg	ttacqggct	tttgcaagaa	ctacaatatt	aaaaggggc	caagctaaaa	780
acagaaaatg	aaaaccaacc	gaaggagatg	tttagactgg	ggagccagat	attgatatgg	840
aatttttta	ttgttagaaaa	gtctgtcgac	ttttttccgg	tgaaatttgg	cttacacgg	900
aaaatgtcaa	ttttaaaaaat	ccaggatcg	atgttttttt	caagccggat	actttctatgt	960
ataactctca	tcgaaatttt	ggtaacaacg	ccatgttca	cagacccaaat	tacattttgt	1020
tcggggataa	ttttgtggcc	cttatgtact	acaacagtgc	tggaaatatg	ggagtttttt	1080
ctggccaacg	atccaaatcg	aatgcgtgg	ttgtacttgc	ggacacaaac	actgaactgt	1140
catatcgct	tttgcgttgc	tctttttttt	acagacccag	ataatctcg	atgttggacc	1200
aggctgtgg	caggatgtat	cccgatgttc	gcattttttt	aaatcccggt	atcgaggatg	1260
aactgcctaa	ttactgtttt	cttcgttggat	gtatggacc	agcggaaaaa	tatcaagggc	1320
ttaaatctta	agacaaatgg	tggaaaaaaat	atgtatgtt	tcttaatatct	aatggaaatag	1380
ccataggcaa	caaccaggct	atggaaattt	atttccaaacg	taacccttttt	agaatttttc	1440
tgtactccaa	tgtggctttt	tacccttccg	atgttttacaa	gtacacggca	actaacatatt	1500
ctctggccca	caacccaaac	acatgtatg	acatggacaa	ggcgtgttgc	tcccatttc	1560
tgttgttgc	atatacatac	atttggccca	gggtgttctt	tgaccaatgt	gacaatgtg	1620
atccattca	ccccacccaa	aatgtgttgc	ttgcgttccg	gttcatgttt	ctgggcaatgt	1680
gtcggtatgt	gcctttccac	atacaatgtc	cttcaaaaaat	tttgcgttgc	aaagaacctac	1740
ttcttcattc	tggtgttccat	acatatatgt	ggaaaccttgc	aaaggatgt	aatatgttcc	1800
tgcacaaatgt	cttggaaat	gaccatggaa	cagatgttgc	tacataatgt	ttcaccagca	1860
tcaatctgtt	tgccacccctt	ttcccccattg	tcacaaacat	acgtttccacc	cttggaaagca	1920
tgcgtcgca	cgataccatc	gateatgtt	tcaacactaa	ccttttttgc	gtaacatgtc	1980
tttacccat	cccccccaat	gcaacacca	ttccaaatcc	catccatctt	cgcaatctgg	2040
caggccatcg	ggggccggcc	tttactatgg	tcaaaaaacaa	ggagacttca	tcttttgat	2100
cagggttgc	tccttacttc	gtatatactg	gtatctttt	ctacctttggat	ggcaccccttt	2160
acccttacca	cactttcaag	aaaggcttca	tcatgttttg	cttccatgtc	agttggcttg	2220
gcaatgtacg	gctgtgtggc	ccaaatgtat	tgttacatca	ggcgtactgt	gacggggaaag	2280
ggtacaaatgt	ggcccaatgt	aaatgtatcc	aaagactgtt	cctgttttgc	atgttttgc	2340
actacaaatcg	ttggatccac	ggctttttca	tccctggatgg	ataacaggat	cgcatgtact	2400
ccttttttgc	aaatcttccat	cctatgtatcc	ggcggatgtt	ttgtatgtt	atttacactg	2460
actacaaatcg	cgtcacccat	ccataccac	acaacaatcc	tggcttttgt	gggtatcttg	2520
cactctatct	gagacaaatgg	ggacacccat	caggccatca	tccatacccg	ctcatcgaa	2580
ctactgttgt	taagagtgtc	accggaaaaa	atgtttctgt	tgacaggacc	atgttggccg	2640
ttcccttcatt	caccaacttc	atgttcatgt	ggggcccttac	cgacccctggga	cagaacatgtc	2700
tctatgtcaa	cttgcacccat	tgccgttgc	tgactttttt	gggtgtatcc	atgtatgtgc	2760
ccacccctgt	ttatctttctt	tttcaatgtt	tcgacgtgtt	cagagtgcac	cagccacacc	2820
gcggcgatc	cgaggccgtc	tacccgtgc	caccgttctt	ggccggcaac	gccaccacat	2880
aagaagccctt	ttgttttttt	caaggcagc	tcgcagcc			2918

<210> 130

<211> 2811

<212> DNA

<213> Adenovirus

<220>

<400> 130

atggccaccc	catcgatgt	cccccaatgg	gcgtatgtc	acatcgccgg	acaggacgt	60
tcggagtatc	tgatgtccgg	tccgttgc	ttcccccggg	ccacagacat	ctacttcgt	120
ctggggaaaca	atgttagaa	ccccacgtgt	gcgccttacc	acgtatgtac	caccgaccgc	180

agccagcggc tgacgcgtg ctttgtccc gtggaccggg aggacaacac ctacttcgtac 240
 aaatgcgt acacgcgtc cggtggcgc aaccgcgtc tggacatggc cagcacccat 300
 ttgcacatcc gccgcgtgt gatcgcccc cttacttca aaccctactc cggcactgcc 360
 tacaacaggc tggtcccaa ggacgcggc aaccctgcg agtggaaaggg ttctgacage 420
 aaaatgcata cttttgggtt agtcgtccat cccgggtta ctggaaadaa gatagaactg 480
 gatggggcgc ctatggat agatccatc tttggactt acacagtaat ttatgtgtat 540
 aaaatttcc aaccagaac caaagtggg aatgacagt ggggtggac caatgtgtca 600
 gaggaaaaaa atggaggcgac agctttaaaag gacactacaa aaatgaaaacc ctgttatgtt 660
 tcattcgcca agcttaccaa caaagaagggt ggtcaggcta actttaaaaaga ttcaaaaaacc 720
 gccgcacca cttcttaacta tgatatacg ctggcttctt ttagacagca aacttattgtt 780
 gctaaactcg atccatggat tgaatgtatc agacaaaatgg ttagacttgc gactccagat 840
 actcatattg tatacaaaac ttggaaacagg gacacacgtt ctgtttttttt tttttgggtcg 900
 caggccatgc ttacacaggc acatccatc ggttccatgg agcaatttat cgggctcatgc 960
 tactacacaa cgtactggca tatogggtgtt ctggccgttc agggcccttca gctgtatgtt 1020
 gtggttgact tgcaagacaa aacactgtaa ctgttccatcc agcttctgt tgactctctg 1080
 ggtgacagaa cccgggtt catgtatggt aatccaggcg tggacagctt tgatccatgtat 1140
 gtgcgcattt tgaaacacca ttgtgtgggg gatgtatggg ctaaatatttg cttttccgtt 1200
 aatgggtgtt gatgtacacaa cacttacccg ggtttaaaagg tttaaacacaa tgcaatgtt 1260
 gaaaatgtggg acaaaggatg caccacagggt agtaatgtatc atgaaatccca tgtaggttt 1320
 ctttttgcgg tggaaatccca catccaccaaa aaccgttggg ggaatccctt ctagtccat 1380
 gtggccttctt atttgtgtca taaaatcaaa tacacacccgg ccaacatccatc cttgtccacc 1440
 aacaccaaca cttacagatg catgacgcg cgggtgggg ccgcctcgct ggtggacggc 1500
 tacattaaacca ttggggccgc ctggcgtcg gaccatggat acaacatggaa tcccttccat 1560
 caccacgcg atgcgggtt ggcgttcccg tccatgtcc tggccaaacgg gcgttccatgt 1620
 ccattccatca tccatgttcc tggaaatttt ttccatccatc agaccccttctt gctctgtcc 1680
 ggttccatca tccatgtgtt gaaatcccgcc aagacgtca acatgtatctt gcaagatgttt 1740
 ctggcaacg accttcgcac acacggggcc tccatccatctt tcaccacggat taacctcttac 1800
 gccacccattt tcccatgtt gcaatccatc gcttccatgg ttagggccat gctgcgcac 1860
 gacaccaatgg acatccatcc tcaacgtact ctccggggcc ccaacatgtt ctatccatcc 1920
 ccggccaaacg ccaccaatcg gcccacatcc atcccttcgtt gcaacttgggc ggcctttccgc 1980
 ggttgcgttcc tcaacgtcgact caagacaaaaaa gagacgcgtt cttttccgttcc ggggttccgc 2040
 ccctacttgc tcaatcttgcgg tccatccatccc taccctcgacg gcacccatctt cctcaaccac 2100
 accttcaaga aggttccatc caccatccgac tttttccgttgc gttttccgttgc caacggccgg 2160
 ctctgtacgc ccaacatcgat tcaatgtatcgg cggccatcgcc acggccgggg atacaacatgt 2220
 gcccacgttcc acatgtaccaaa ggatgggtt ctgttccatcc tggccggccat ctacaacatcc 2280
 ggcttccagggtt gttttccatgtt gcccggggcc tcaacaggacc gcatgtatctt cttttccgttcc 2340
 aacttccacg ccatgtccgg ccagggtgtt gacgggttta actacaaggaa tcaacaggcc 2400
 gttaaccatgg tccatccatca caacatcgat ggttccatgtt gataccatccgc gcccactatgt 2460
 cgccaggatccgg agcccttaccc cgccaaatccatcc cccatccatccgc tcatccgcac gaggccgtt 2520
 accacgttcc cccaaaaaaa gtttccatgttgc gacgggttcc tggccgcattt ccccttcc 2580
 agcaatgttcc tggccatgtgg tggccatcccg gacccatccgc acacatgttcc ctatgttac 2640
 tccggccatcc gcttagatcat gatatttgcgg tggaccccca tggatgagcc cacccttctt 2700
 tatgtgttgc tcaagatgtt ccacgttgc tggatgttcc acggccacccg cgggttccattt 2760
 gaggccgttcc accttcgcac ccccttccatca gccggtaacg ccacccatata a 2811

<210> 131

<211> 2850

<212> DNA

<213> Adenovirus

<220>

<400> 131

atggccatcc catcgatgtt gccccagggtt gcatatcatgc acatcgccgg acaggatgtt 60
 tcggatgttcc tggatccggg tctgtgtccat gtcggccatcc gccacagacac ctacttcataat 120
 ctggggaaaca agtttaggaa cccacccatgtt ggcggccatcc atgtatgtatc caccgaccgc 180
 agtcacgcgg ttagtgcgtt ctttgtgtcc gttggccgggg aagacaatatc ctacgcataat 240
 aaatgttccatcc acacccatggg tgggtggccatcc aacagatgtt tggatgttgc cagactttt 300
 tttggggatcc ggggtgtgtt ggtatggatcc cttacttgc tttttccatccgc tggccatgtt 360
 tacaactcg tggcccttcaatccgc tttttccatccgc tttttccatccgc tggccatgtt 420
 aaaaatggatcc atggggatcc tgacgaagag gaagagaaaaaa atttccatccgc ttatctttt 480
 ggaatgttcc cttacttgc tttttccatccgc tttttccatccgc tttttccatccgc tttttccatccgc 540
 ggttccatccgc tttttccatccgc tttttccatccgc tttttccatccgc tttttccatccgc tttttccatccgc 600
 gacacccatcc gttggatgttcc gttggatgttcc gttggatgttcc gttggatgttcc gttggatgttcc 660
 ggttagatgttcc taaaaggccatcc gttggatgttcc gttggatgttcc gttggatgttcc gttggatgttcc 720
 actaaatgttcc aaggccatcc gttggatgttcc gttggatgttcc gttggatgttcc gttggatgttcc 780
 gttagatgttcc tttttccatccgc tttttccatccgc tttttccatccgc tttttccatccgc tttttccatccgc 840
 cccaaatgttcc tttttccatccgc tttttccatccgc tttttccatccgc tttttccatccgc tttttccatccgc 900

tacaaacactg	gtacttcaga	agaagaatctt	Catgcataatc	tggggcaaca	atctatgc	960
aacagaccca	actacatttg	cttagagat	aactttatg	gacttatgt	ctacaacagt	1020
acttgcacaa	tgggagtgt	ggcaggtaa	gcatcccaa	tgaatgcgt	ggttgactt	1080
caggacagaa	acacaaga	atcatatca	ctactgt	acttctgg	tgacagaac	1140
agatacttca	gcatgttga	tcagaacgtc	gatacgat	atccgtatg	gcgcattatt	1200
gaaaatcttgc	tgatggaa	tgacttccc	aactactgt	ttccatgtt	ttggatgtgg	1260
gtaccaataat	taqtgttaca	aataattgtt	ccaaacggac	agggtgcaga	ttggaaqaag	1320
cctgcataata	atggaaacaa	tgaaatttgg	caaggaaatc	tcttgcct	ggaaatattaa	1380
cttcaacgta	atcttcgttg	aaggtttt	tatccaatg	tggtctgt	tctcccgac	1440
tcctcaataat	acacccccagc	caatgtcact	cttccaacta	acaccaaac	ttatgactat	1500
atgaatggcc	gggtgtttcc	ccatccccc	gtggatatact	acgttaaacat	ttggccgac	1560
tgttctttgg	atggcatgtt	caatgtca	ccctttaacc	atccacccaa	cgttgtcc	1620
cgtatccccc	ccatgttccc	ggcaatgtt	cgatgtcg	ttttccacat	tcaatgtcc	1680
cggaaatttt	ttgttgttga	gaacccgtt	ttttatccgg	gttcttacat	ctacgagtgg	1740
aacttcagaa	aggatgttga	catggtcgt	catggttccc	ttggtaatga	tctccgggtc	1800
gatgttgc	caatgttgg	tacacgtat	aacttctat	ccacccctt	ccccatggcc	1860
cacaacactg	ctttccaccc	tgaaacgtat	ttggccaaat	acaccaatga	tcaatcatte	1920
aatgactact	ttttctgttc	caatgtc	ttccatccat	cgcccaacgt	taccaacgtt	1980
ccatcttca	ttttccatctg	caactgggg	ttccatccat	ttttccatcc	caccagactc	2040
aaaaccaagg	agactccctt	tttggatca	gggtgttgc	cctactttgt	ttactctgtt	2100
tctatccat	acttgcgtt	tacccatcc	tttaaccaca	tttcaagaa	agttccat	2160
atgtttgact	tttcgttg	ctggcctgtt	atagacat	tgtaatgtt	aatgtgtt	2220
gaataatcc	gcacatgtt	ttgggaaagg	tacaatgtt	cccaatgtt	catgaccaat	2280
gactgttcc	ttgttccat	gtttgcac	tacaacat	gataccat	ttttccat	2340
Cttcgagggtt	acaaaggatc	catgtactt	ttttccat	actttccat	catgatgtt	2400
cagggttgg	atggatattt	tttcaaaag	tataaagtc	ttccgttacc	ctaccat	2460
aataacttgc	gttttgttgg	tttacatgtt	ccttacat	gtcagggtt	agcgttccat	2520
gtaacttac	catatccccc	aattgttgc	actgttgc	ccatgttgc	ccggaaaaaaa	2580
ttctctgtgg	acaggacat	gttgcgcac	ccatcttca	gcaacttcat	gttccatgtt	2640
gcccttacag	ttttgtggaa	gaatgttgc	tatgttca	ccggccatgc	gttggatcat	2700
acttttgagg	ttgtatccat	ggatgttgc	acccctgtt	attttctttt	cgaatgttcc	2760
gacgttgc	gagtgcacca	gccaacccgc	ggcgttcat	aggccgttca	cctgcgcaca	2820
ccgttccgc	ccggcaacgc	caccacataa				2850

<210> 132

<211> 2907

<212> DNA

<213> Adenovirus

<220>

<400> 132

atggcttacc	tttcgtat	ggcccgatgt	tcttacatgc	acatctcg	ccaggacgc	60
tcggagttacc	tgagccccgg	gttggtgc	tttgcgcg	ccaccgc	gtacttc	120
ctgaaataaca	aggatggaa	ccccacgtt	gcacccat	acgacgttac	cacagaccc	180
tcccgatgtt	tgacgttgc	tttcatcc	gtggacccg	aggataccgc	gtacttc	240
aaagccgtt	ttttccatcc	ttgggtgtt	aaectgttgc	tttgcgttgc	tttccatgtt	300
tttgcatcc	ttgggtgttgc	ggacacgggg	cctacttta	ttttccat	ccggcacttgc	360
tacaacgc	tagtccca	gggcgttct	aactctgt	agtggaaaca	aaccgaat	420
agccgggg	catgttgc	ggatgttgc	ggggatgtt	aaatgttgc	agggaaagaa	480
gaagacaaa	acgttgc	ttgggttact	ttttccat	ttttccat	ccaggcttct	540
tttgttgg	aaacaaatcc	aaaacggatc	tttccat	ttttccat	ttttccat	600
caagtttacaa	ttgtatccat	ttttccat	ttttccat	ttttccat	ttttccat	660
cagtggaa	ttttccat	ttttccat	ttttccat	ttttccat	ttttccat	720
atgaaaccat	ttttccat	ttttccat	ttttccat	ttttccat	ttttccat	780
ctgttccgg	ttttccat	ttttccat	ttttccat	ttttccat	ttttccat	840
actacccctt	ttttccat	ttttccat	ttttccat	ttttccat	ttttccat	900
gaagatgtt	ttttccat	ttttccat	ttttccat	ttttccat	ttttccat	960
gaaaatccat	ttttccat	ttttccat	ttttccat	ttttccat	ttttccat	1020
ttcaggagca	ttttccat	ttttccat	ttttccat	ttttccat	ttttccat	1080
gttgggttgc	ttttccat	ttttccat	ttttccat	ttttccat	ttttccat	1140
tccatccat	ttttccat	ttttccat	ttttccat	ttttccat	ttttccat	1200
caggctgt	ttttccat	ttttccat	ttttccat	ttttccat	ttttccat	1260
gaatgttgc	ttttccat	ttttccat	ttttccat	ttttccat	ttttccat	1320
attaatggca	ttttccat	ttttccat	ttttccat	ttttccat	ttttccat	1380
acttttgc	ttttccat	ttttccat	ttttccat	ttttccat	ttttccat	1440
aatggcaacc	ttttccat	ttttccat	ttttccat	ttttccat	ttttccat	1500
ctaaaaataca	ttttccat	ttttccat	ttttccat	ttttccat	ttttccat	1560

aacaacgtag tggtggctcc cggccttgc gactgtcata ttaaccttgg ggccgcgttg 1620
 tctctggact acatggacaa cgttaatccc ttaaaccacc accgaatgc gggccctccgt 1680
 ttcgtccca ttgtttggaa aacccggccg tacgtccctt ccatacattca ggtgccccaa 1740
 aagttttttgc cttataaaaaa cttcccttcic tcggcaggct cataatacata tgaatggac 1800
 ttccagaagg atgttaaacat gtttgcagc agctctcg gaaacgatct tagatgtac 1860
 gggcttagca taatggttga cagcatttgtt cttacgcga cttcttccc catggcccaa 1920
 aacacggccctt ccacggtaga agccatgtc agaaaatgaca ccaacgacca gtcccttaat 1980
 gactactttt cccgcggcaa catgtatcc cccataccgg ccaacggccac caaecgtgcc 2040
 attcctccatc catcgccaa ctggcggca tttcgccgtt gggcccttcac acgcttgaag 2100
 acaaaggaaaa ccccttcctt ggaaatcgcc tacgaccctt actacaccta ctctggctcc 2160
 atatacatac ttacgggaac cttctatctt aatccacact ttaagaaggt ggccattacc 2220
 tttagactctt ctgttagctg cggggggcaac gacccgtcgc ttactccaa ttagtttgag 2280
 attaaacactt cttgttgcgg ggagggtcc aacgttagctt agtgcacat gaccaaggag 2340
 tggttcttgc tgtagatgtt ggccaactac aatattggctt accagggtttt ctataticca 2400
 gaaagtcata agggccgcat gtactgtcc ttccagaactt tccagcccat gaggccgcaa 2460
 gtgggttgcg actataataa caaggagtagt cagcgggtt gaatttttca ccagcataaa 2520
 aactcaggat tcgttagctt ctcgtccccc accatgcgcg aggacacggc ttacccccc 2580
 aacgtgtccctt accactaat aggccaaaccc ggggttgcata gtatcccca gaaaaggtt 2640
 ctttggcgtat gcacccctttt ggcgcataccca ttctccagta actttatgtc catggccqca 2700
 ctacacagac tggcccaaa cttcttcattc gccaactccg cccacgcgtt agacatgact 2760
 tttaggttgcg atttccatggta cgacccccc ttctttatg tttgttttgaa agtcttttgac 2820
 gtggccgttg tgccacccggc gcacccggcc gtcatacgaga ccgtgttactt ggcacacccc 2880
 ttccggccgg gcaacggccaa aacataaa 2907

<210> 133

<211> 2757

<212> DNA

<213> Adenovirus

<220>

<400> 133

atggccactc cttcgatgtt gcccgcgtgg tcttacatgc acatcgccgg tcaggatgcc 60
 tcggagttc tgactccccc ttctgttgcata ttccgcggcg ccacggacac ctacttcacc 120
 ctggaaacaa agtttagaa cccacgtgt gcttccatctt atgtgttac caccgtatcc 180
 tcgcgcgtc tgacgcgtgg ttttgcgtcc ttgtatcggg agataactac ctacttcacc 240
 aaggctcgcc ttacgcgtgg tttttgttgc aaccgcgtgt tagacatggc tagtttttac 300
 tttagatttc gagggttactt ggatcggtt cccatgtttt acggccatttc cggaaacccggc 360
 tacaattttt tggccaccaa aggccgttcc aatgtttcac aatggtcaga taacgctaag 420
 cttaaatccctt ttgtcggg gccgttatcc aacgcacacta tcacccggc cgatggattt 480
 aaagttggaa gagacacccg ccaggccggc ggccgggtt atgccaacaa aactttatcg 540
 ccagacggcc aatgttggcc aatgttgcgtt aacccacgtt ttgaaaacgcgt taaaatggc 600
 gggggggcat taaaacaaac cactgttgcgtt cggccgtgtt atgtccctta cgttcgttca 660
 accaaacgaa acggaggacaa atccaaagggtt gacaaatccgtt aactttaagttt ctttgcattca 720
 gctaataatcg cggaaacac tgcgtcaatgtt gtgttctata cggaaagacgtt aaacctttgaa 780
 atggcagaca cgcattttgtt gttttaagcctt actgttacca atggaaacat tgcgttgc 840
 tcgtgttgcg gaccaacggc agccgttacat agacaaatccgtt aacattgtt cagagataat 900
 ttatattggcc tgatgttgcata caacatgtt ggcacatgtt tgatattggc cggccgttgc 960
 tcccaactt acgcgtatgtt agacccgttca gagacaaatccgtt aacccatgtt cggccgttgc 1020
 atgctgtatg ttttggggaa cggaaacccggc tttttttctt tggtggatcc cggcgttgc 1080
 aqttacgacc ctgacgtttcg ctgttattgtt aatccacgggg tagaggatgtt actacccaaat 1140
 tatgtttttt cttcttagcg agatgttgaa ataaataaaatccgtt aacaaaggcat taaggccat 1200
 aacggggaggat ggggttgcgtt gacttgcggc aacactgttca tggtggacaaat cccatcatggc 1260
 atttggaaata tggccgttata gggaaatattt ttgcgggttcc atttgttggag aagcttcttgc 1320
 tactcaatgtt tggccgttata cttccatggac gactttttatccgtt aacccatccggg aacataaaaa 1380
 ctacctgtata acaaaacac ctacgtgtac atgaaacggcc gtgtgtactgc cccgggggtt 1440
 gtggataccat atgtcaatgtt cggccgttgc tggttccccc atgtgttggat taaatgttac 1500
 ccttttaacc accaccggaa ccgggggttgc cgttacatgtt ccatgttgcgtt aggccataatggg 1560
 agatgtttgc ttttccatcat tcgggttgc cttttttttt ttgcgtatcg aatattttgttgc 1620
 ctgttgcggcc gttccatcatc ttccatgttgc aactttttatccgtt aacccatccggc ctttttttgc 1680
 cagacgcacat tggggaaatgtt tccatgttgc gacggccgtt ccgttgcgtt tgacaaacattt 1740
 gcccgtatgtt ctatctttt tccatgttgc ctttttttgc ctttttttgc ctttttttgc 1800
 tttaaagaaatgtt acaccaacggc ccgttgcgtt aacccatccgtt ttttttttgc aacccatccgtt 1860
 tatttttttttccatcatc ctttttttgc ctttttttgc ctttttttgc ctttttttgc 1920
 gcattttagat gtttttttgc ttttttttgc aacccatccgtt aacccatccgtt ctttttttgc 1980
 ggttttttttgc ctttttttgc ttttttttgc aacccatccgtt aacccatccgtt ctttttttgc 2040
 cttaaaccaca ctttttttgc aacccatccgtt aacccatccgtt ctttttttgc ttttttttgc 2100
 aatgaccgtt tgtaaaccatc aatgttgcata atgttgcata ctttttttgc ttttttttgc 2160

tacaatgtgg	ccaaattgca	tatgactaa	gattgttcc	taatacaat	gcttagtc	2220
tacaacattg	gataccagg	ttttacatt	ccagagact	acaaggacc	catgtatct	2280
ttcttttagaa	atttcgc	catggatgg	caatgttg	ataccacaga	atataa	2340
tacaaaaaa	taaccgt	tttacat	acaatc	gattcg	ataccctgg	2400
cccaactatgc	ggggggaca	agcttaccc	gccaactatc	cctaccc	tataggc	2460
acagtgtgg	aaggatcac	acagaaaa	tttctatgc	atctgttat	gtggcgatc	2520
ccatTTTCTA	gtactatcat	gtctatgggg	gcgcta	atcttgc	aatatgtc	2580
tagcaaaact	ccccatgc	tctagacat	acatttgagg	ttgatccaa	ggatgagct	2640
acccttctt	atgttttt	tgaagt	gacgttgtac	gcattacca	gcccacacc	2700
ggcgtattt	aagcggtct	cttgcgac	cccttc	cgggtaa	taccacc	2757

<210> 134

<211> 2895

<212> DNA

<213> Adenovirus

<220>

<400> 134

atggcttacc	tttcgtat	gccgcgtgg	tcttacatgc	acatctcg	ccaggacgc	60
tcgggttacc	ttagccccgg	gtcggtcg	ttcgccccgg	ccaccg	gtacttc	120
ctgaataaca	atgttagaa	ccccacgg	gcgcctac	acgacgt	cacagac	180
tcttcgtt	tgcgttgc	gttatttccc	gtggacccgg	aggatacc	atactc	240
aaggccgg	ttaccatgc	gttgggtgc	aaccgtgt	ttgatccat	ttccacat	300
tttgcattc	tgccgtgt	ggacgggg	cccatctt	agccctact	cggcact	360
tacaacgtc	tagcccca	aggcgctcc	aattctcg	atgtggaa	agaaga	420
actcaggaaa	ttgtgtaa	acttgaat	gaggagg	caaggagg	ggaggc	480
gaggagg	aaagcacca	actgtatc	aagggtt	agactc	atagc	540
gttcccttgg	caggggaaa	aatttgc	atgttt	aatatgtt	tgatccat	600
actgaaggaa	atccagg	tccgtatccc	acttata	ctgaatcc	gttggagat	660
tcttcgtt	atgttgc	agcaatc	tctgggg	gagta	aaagact	720
cccatgaaac	catgtatcc	atcgatqc	agacctaa	ataaaaatgg	gggtcaag	780
atactatgt	caaaacaa	agggtctc	gatgtt	ttgaaatgc	gtttttgc	840
cccttggca	ttcgat	tgaaagaaat	gtgttgc	caagtat	tttgatc	900
gaggatgtt	atgttggaa	tccgtat	cacatcc	acaacacc	caaaactgt	960
aaaaactcta	agctgtt	gggttcaaa	gcaatc	acagac	tttacatgc	1020
ttttagggaca	atttttat	tccgtat	tacaac	ctgttacat	gttggat	1080
gttggacaag	catcaca	aatgtcg	gttagact	aggac	cacagac	1140
tcatatca	tttgcgt	tttattgc	gatagaac	gatact	catgtt	1200
caggctgt	acgttgc	tccgtat	agatgtt	aaaatcatgg	aactggat	1260
gagggtccaa	attactgtt	ccacttgc	ggtagatgg	taactgc	ctacc	1320
ataaaaatca	acggaaac	tatgttca	actgttgc	aaaatgtac	tttttgc	1380
ctgtatggaa	taggtgtgg	aaacaa	cccttgg	ttaaatctt	ttccaa	1440
tggagaatt	tccctatc	caacatt	ctgttac	ctgaca	aaaatact	1500
cctacaatgt	ttggaaat	tcccaac	aatccat	attatgtaa	caagcgat	1560
gttggctcc	gttgggttgc	ttgttacat	acccgttgg	cggttgc	atttggat	1620
atggacaatc	taaccatcc	tacatcat	cgaaatgc	gcttacgt	ccgttccat	1680
tttgcgttgc	acgtgttgc	ctgttgc	catatcc	tttttgc	tttttgc	1740
atttggaa	tccctatc	ggccggctt	tacatcc	actgttgc	tttttgc	1800
gttacatgtt	tttgcgttgc	ttcccttgc	aaacat	gatgttgc	aggc	1860
aaatttgc	gttgggttgc	tttgcgttgc	tttttgc	ttcccaat	cacc	1920
acgttgc	tttgcgttgc	aaacat	aaacat	tttttgc	tttttgc	1980
ggcccaaca	tttgcgttgc	tacatcc	aaacccat	acgttgc	tttttgc	2040
tcgcgtca	ggccggctt	ccgggttgc	ggccgttac	gcttta	aaaggaa	2100
ccatccctgg	tttgcgttgc	tttgcgttgc	tatata	tttttgc	tttttgc	2160
gacggaa	tttacat	tttgcgttgc	tttgcgttgc	tttttgc	tttttgc	2220
tttagcttgc	tttgcgttgc	tttgcgttgc	tttgcgttgc	tttttgc	tttttgc	2280
tttgcgttgc	tttgcgttgc	tttgcgttgc	tttgcgttgc	tttttgc	tttttgc	2340
cagatgttgc	tttgcgttgc	tttgcgttgc	tttgcgttgc	tttttgc	tttttgc	2400
gaccgtat	tttgcgttgc	tttgcgttgc	tttgcgttgc	tttttgc	tttttgc	2460
acccaaatca	aggtatca	tttgcgttgc	tttgcgttgc	tttttgc	tttttgc	2520
tttgcgttgc	tttgcgttgc	tttgcgttgc	tttgcgttgc	tttttgc	tttttgc	2580
ccgtttagat	tttgcgttgc	tttgcgttgc	tttgcgttgc	tttttgc	tttttgc	2640
acccttgc	tttgcgttgc	tttgcgttgc	tttgcgttgc	tttttgc	tttttgc	2700
ggccaaaaac	tttgcgttgc	tttgcgttgc	tttgcgttgc	tttttgc	tttttgc	2760
ccatgttgc	tttgcgttgc	tttgcgttgc	tttgcgttgc	tttttgc	tttttgc	2820
cacccatgc	tttgcgttgc	tttgcgttgc	tttgcgttgc	tttttgc	tttttgc	2880
aaacccatca	tttgcgttgc	tttgcgttgc	tttgcgttgc	tttttgc	tttttgc	2895

<210> 135
<211> 1110
<212> DNA
<213> Adenovirus

<220>

<400> 135

ttttgacatc	cggccgtcc	tggaccgcg	tcccgctt	aaaccctact	cggcacggc	60
ttacaacagc	ctggccccca	aggcgcccc	caatcccagt	cagtggaaac	aggaaaaaac	120
aggagctgtt	gtgacccaaa	atcaaaaaga	aactcgact	tacggcttag	ccggcactgg	180
cggataaac	attacaaaag	aaggacttc	gattgaaatt	gatgaaacta	aagaagatcc	240
aaacaacaaa	atttgcac	ataaaaaatt	ccacggggaa	ccacaaatag	ggggaaataaa	300
ttggcggatc	aaataatgtt	tctatggccg	cagactctt	aaaaaggaaa	Ccaagatgaa	360
accatgtcat	ggcttatttg	ccagaccaac	taataaaaaa	ggaggcgg	ctaaagtttt	420
aaccactgaa	gatggtcaac	ctacagaaaa	tttgacata	gacttggctt	tcttgatat	480
tccacaatgt	ggggaaatgg	acaatcttga	tccagatatg	atcttgtatg	ccgaaaatgt	540
aaatcttagaa	acacccgtaca	cacatgtatg	ttataaccca	ggaacggatg	atgcacgtt	600
tgcgcgtac	cacccacac	agtcacatgg	aaacatcgcc	aactacatcg	gtttcaggga	660
caacccatgt	ggacttattgt	attacaacag	cactgtcaac	atgggtgtt	tggtgttca	720
ggcccttcag	ttaaatgtt	ttgtcgactt	gcaagacaga	aataccggac	tgtcttatca	780
gctttttat	gattcttgg	gtgacgggg	cagatctttt	agatatgtgg	acttcggctt	840
ggacagctat	gatccatggat	tcaggatcat	tgaaatcat	gggtggagg	acgaacttcc	900
aaatattgtt	tccccatgtt	atgttccgg	tccaaatgtc	acatccaagg	gttggaaacct	960
agataatgtt	caaggcttcaa	atgttataatg	ggaaaaggac	gagaaggatg	ctgttcaaaa	1020
ccaaatgtt	aggggcaata	tttgcacat	ggagatiaac	ctccaggcca	acctgtggaa	1080
gagggtttcg	tactcgaaac	tgcccttgg				1110

<210> 136

<211> 2769
<212> DNA
<213> Adenovirus

<220>

<400> 136

atggccaccc	cctcgatgt	gcccgaatgg	tcttacatgc	acatcgccgg	gcaggacgcc	60
tcggagtacc	ttggcccccgg	cttgggtcgag	ttcgccccgt	ccacccgatac	ctacttcagc	120
ctggggaaac	agtcagaaaa	cccccccggt	gtccccccac	acgtatgtaa	cacagacgg	180
tcgcacgcac	tgacgtcg	ttctggcc	gtgcacggcg	aggaaacccg	ctacttttac	240
aaatgtcgcc	ttacgtcg	cgtggccgg	aaccgggtt	ttggacatggc	cagcacccat	300
tttgacatcc	ggccgtcg	ggatctgtt	cccaatcttta	accccttattc	gggcactgtc	360
tacaacttcc	ttggccccc	agggtgttcc	aatcttgcatt	agttggacaaa	ccaaaaacaaa	420
acaaacttcc	tttggaaac	tcccttata	ggacaaaaaa	tcaccaatca	gggcgtgcaa	480
gtgggtcg	acttcaacaa	tcgcgtgt	tttgcgata	aaacgttcc	acccggaccc	540
Caatgggggg	agacgtcaat	gaaatattaa	ccatgtcaat	acgtcgccgg	aagaatacta	600
aaaacaaacca	ccggccatca	ggccatgtt	gggttacatcg	ctagaccaac	aaaacaaaaa	660
ggagggtttcg	ccaaatgtgtt	aaaaatgtg	gacaaatcga	ccacaacaaac	aaacgttaggt	720
ttaaactttt	tttaccaact	ctgttttttt	gtttttttttt	cacccaaatgtt	ggtttctgtac	780
agcgaatgtt	ttaactttaga	agcgtccgt	acccacccgt	ttgtttttttt	agatgttcaac	840
ggccacaatgg	ccggatcttt	actggggatc	caggccgtcc	ccaaatgttca	taatattttt	900
ggtttttttt	acaatcttca	tttttttttt	tatctttttt	ccactgttca	catggggatgt	960
ctggccgggg	aaatgtttttt	gtctcaacgg	gtgtttttttt	taatgtttttt	aaaacacgggt	1020
ctgtttttttt	atgttataatgt	tgacgtttt	ggggatcgaa	gtcgatfactt	ttttatgttgg	1080
aaccaggcgt	ttggacagctt	tgaccccgac	gtgagaattt	tttggaaatca	ttggcgtggaa	1140
gacgatgtcc	ccaaatgttt	tttttttttt	aatggggatgt	gaatatcttad	cagtaccaat	1200
ggcgatggaa	ctgacatgg	aaactatgg	tctgtttttt	atacagacgt	ctcaacaaac	1260
aacaaatgtt	ccatggccaa	ttgttttttt	atggggatgt	atctttttttt	taatctttttt	1320
agaatgtttt	ttgtactcaaa	ttgttttttt	tacttttttt	actttttttt	aaaatcccccc	1380
gataacatcc	tttttttttt	caaaaaat	acatgttcaat	acatgttcaat	ttgggtttttt	1440
gtcccccgg	cccttggat	atatcggttt	atggggccgt	gtgtttttttt	agaccccccatt	1500
gacaacgtt	atcccctttt	ccacccggc	aatgttgcgt	ttcgcttaccc	tttcatgttct	1560
ctgggttacg	ggccgttacgt	ggccccc	atccatgttca	cccaaaaaat	tttgcgttcc	1620
aaaaatctcc	ttgttctgtcc	ccgggttccat	acccatgttca	ggaaatctcc	gaaggatgtt	1680
aacatgtttt	tccaaacgg	ttcttttttt	atccatgttca	ttccatgttca	cagcgttccagg	1740
tttggacagca	tttacatgttca	tttttttttt	ttccatgttca	ttccatgttca	cccttccacc	1800
tttggacagca	tttacatgttca	tttttttttt	ttccatgttca	ttccatgttca	ttccatgttca	1860

gcaaacatgc ttaccccat acccgccaa cgtactagcg tgccccattc tattcccttc 1920
cgaatgggg ctgttttcg ggggtggagt ttactagac taaaaactaa agaaacccc 1980
tctttggggg ccgggtttgatccatatttc acctacttg gctccgtccc atacttggt 2040
ggcacccctt acctaaccac cactttaaa aagggtgtccg ttatgttgcg ctccctgtg 2100
agctggcctg gtaacgaccg actactact cccaacgagt ttgaatacaa acgaaccgtg 2160
gatggggaaag gataacaacgt ggctcaatgt aacatgacca aggactgggtt cctcatacaa 2220
atgtctcgtc actacaataat tggttccatcgg aatgggttccatcgg taccagaaaatc 2280
aggatgtact ctttttccg aaacttccaa cccatgagcc gccagggtgg agacactacc 2340
acctacacgg agtatcagaa tgaactctc cttttccagc ataataacttcc tggctttgtt 2400
ggatacatgg gacccgtcat acggggggaa caagtttacc ccggccaaacttccatcc 2460
cttattggtc agacggccgtt accaaggctg actcagaaaaa aattttttttt cgatgttacc 2520
atgtggcgtc ttcccttttc cagccaaacttccatcgg ttttttttttttggatggatgggg 2580
caaaacatgc tgatcgccaa ttcccgccac ggctcgacaa tgacttttggatggatgggg 2640
atggatgagc ccacacttctt ctatgttgcg ttccgaatgtt tcgacgttgtt ggcgtatccac 2700
cagccgcacc gcggcgcatc cgagggcgatc tacctgcgtt ccgcgttctc ggccggtaac 2760
gcaccacaca

1/5

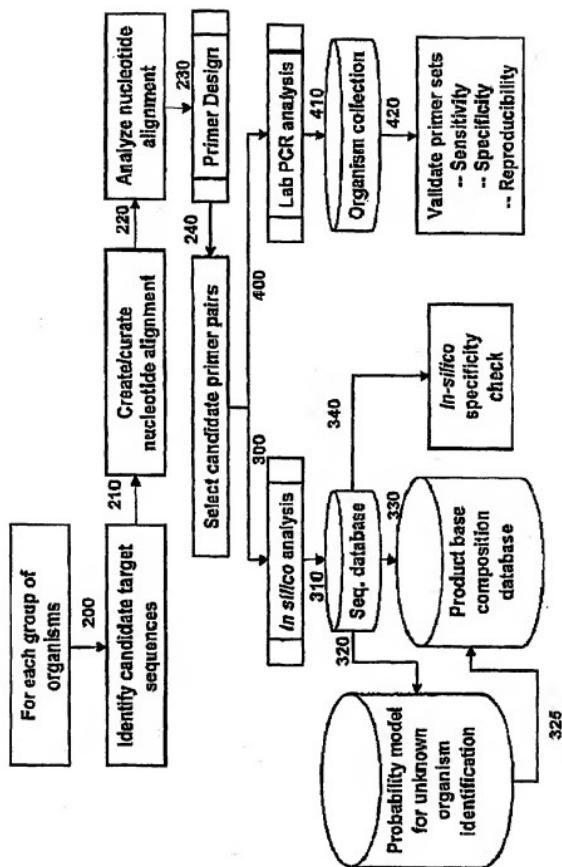


Figure 1

2/5

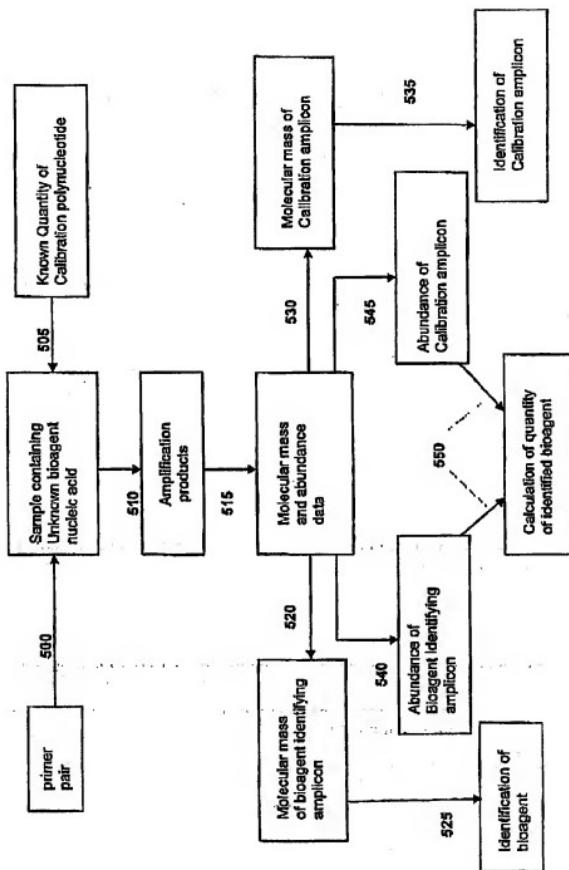
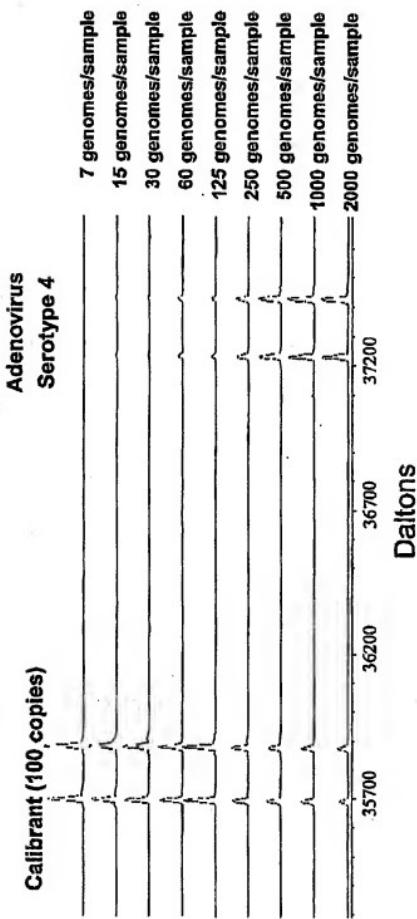


Figure 2

3/5

**Figure 3**

4/5

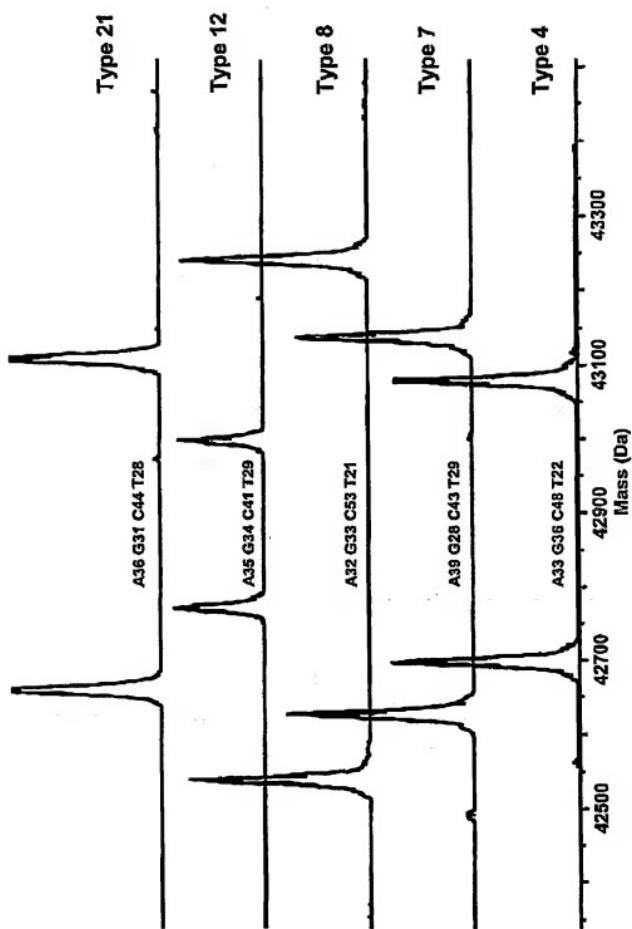


Figure 4

5/5

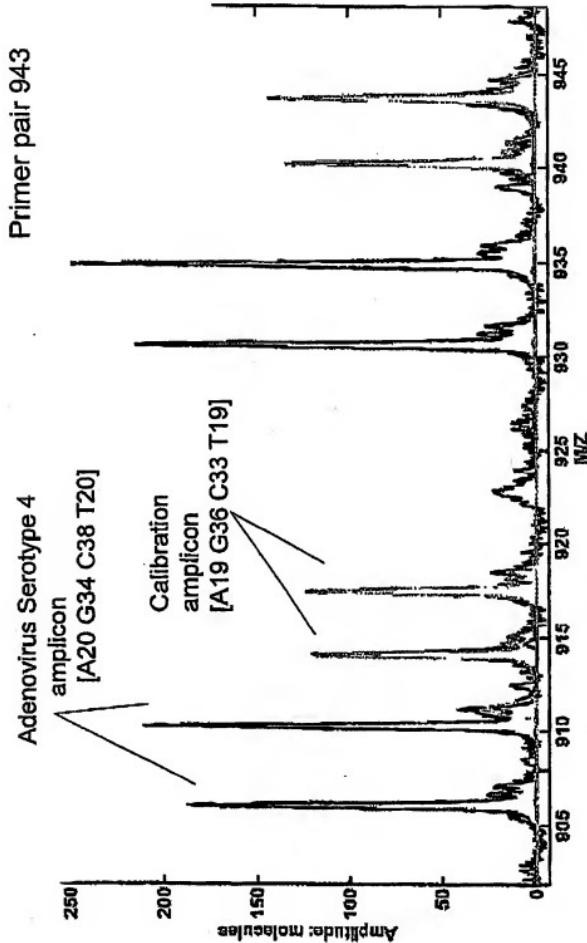


Figure 5

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
2 August 2007 (02.08.2007)

PCT

(10) International Publication Number
WO 2007/086904 A3(51) International Patent Classification:
CI2Q I/68 (2006.01) *CI2Q I/70* (2006.01)

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SL, SM, SY, TI, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(21) International Application Number:
PCT/US2006/014178

(22) International Filing Date: 12 April 2006 (12.04.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/671,003 13 April 2005 (13.04.2005) US

(71) Applicant (for all designated States except US): ISIS PHARMACEUTICALS, INC. [US/US]; 1896 Rutherford Road, Carlsbad, CA 92008 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): HALL, Thomas, A. [US/US]; 5239 Wohlford St., Oceanside, CA 92056 (US). SAMPATH, Rangarajan [US/US]; 12223 Mannix Road, San Diego, CA 92129 (US). BLYN, Lawrence [US/US]; 27892 Perales, Mission Viejo, CA 92692 (US).

(74) Agents: CASIMIR, David, A. et al.; Melden & Carroll, 101 Howard Street, Suite 305, San Francisco, CA 94105 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
13 September 2007

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2007/086904 A3

(54) Title: COMPOSITIONS FOR USE IN IDENTIFICATION OF ADENOVIRUSES

(57) Abstract: The present invention provides compositions, kits and methods for rapid identification and quantification of adenoviruses by molecular mass and base composition analysis.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/014178A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68 C12Q1/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, Sequence Search, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/060278 A (ISIS PHARMACEUTICALS INC [US]; ECKER DAVID J [US]; GRIFFEY RICHARD H [) 22 July 2004 (2004-07-22) page 7, line 18 - page 8, line 20; claims 41,42; figures 17,21 page 30, line 10 - line 15; claims; example 17 page 18, line 3 - line 6 page 22, line 15 - line 17 -----	1-20
X	WO 96/29431 A (SEQUENOM INC [US]) 26 September 1996 (1996-09-26) page 21, line 34 - page 22, line 24; claims; figures 24,25 page 23, line 6 - line 19; example 5 ----- -/-	1-20

 Further documents are listed in the continuation of Box C. See patent family annex.

Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the International filing date
- *L* document which may throw doubts on priority, claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Z document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the International search report

17 July 2007

26/07/2007

Name and mailing address of the ISA/

European Patent Office, P.B. 5616 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Pilat, Daniel

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/014178

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YAO Z P ET AL: "Mass Spectrometry-Based Proteolytic Mapping for Rapid Virus Identification" ANALYTICAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, COLUMBUS, US, vol. 74, no. 11, 1 June 2002 (2002-06-01), pages 2529-2534, XP002980424 ISSN: 0003-2700 -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2006/014178

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
WO 2004060278	A	22-07-2004	AU	2003297687 A1		29-07-2004
			CA	2508726 A1		22-07-2004
			EP	1578399 A2		28-09-2005
			JP	2006516193 T		29-06-2006
WO 9629431	A	26-09-1996	AT	232558 T		15-02-2003
			AU	722218 B2		27-07-2000
			AU	5365196 A		08-10-1996
			CA	2214359 A1		26-09-1996
			CN	1202204 A		16-12-1998
			DE	69626196 D1		20-03-2003
			DE	69626196 T2		11-09-2003
			DK	815261 T3		10-06-2003
			EP	0815261 A2		07-01-1998
			ES	2194985 T3		01-12-2003
			JP	11508122 T		21-07-1999
			JP	2006271382 A		12-10-2006
			PT	815261 T		30-06-2003
			US	6043031 A		28-03-2000